THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 122 JUNE 1, 1938 No. 3

# THE EFFECT OF THYROXIN ON THE CARBOHYDRATE METABOLISM OF HYPOPHYSECTOMIZED RATS[1]

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Received for publication January 29, 1938

The metabolism of carbohydrate in hypophysectomized rats differs from that in normal animals. During fasting, carbohydrate stores are not maintained as in the normal, first the liver glycogen and blood sugar levels falling rapidly, then the muscle glycogen also showing a marked decrease (1, 2). When hypophysectomized rats are fed glucose, they deposit a smaller proportion of the absorbed carbohydrate as glycogen, and apparently oxidize a greater part than do normal rats (3). The rate of absorption of glucose from the intestine is much lower in hypophysectomized than in normal rats (4), and the metabolic rate is also decreased by about one-third. Whether either the low absorption rate or the low B.M.R. may be in any way responsible for the other changes observed in the carbohydrate metabolism of hypophysectomized rats has not been known.

Recently, Althausen (5) has found that thyroxin treatment is capable of producing a marked increase in the rate of absorption of glucose and of other sugars by normal rats. In view of the well-known thyrotropic function of the anterior pituitary, it seemed worth investigating whether thyroxin could affect the absorption rate of glucose as well as the metabolic rate of hypophysectomized rats; and if so, whether the disposition of the absorbed carbohydrate were affected by these changes.

PROCEDURE. Young male hypophysectomized rats, weighing 150 to 180 grams, were used two weeks post-operative. They were each given 10 or 20 gamma of crystalline thyroxin daily for 10 days, the thyroxin being dissolved in a small amount of very dilute NaOH solution and injected intraperitoneally. At the end of the injection period, the rats were

[1] Aided by grants from the Board of Research of the University of California and the Rockefeller Foundation of New York City.

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fasted 18 hours and then fed glucose in an amount sufficient for maximal absorption during the ensuing four hours. The disposition of the fed carbohydrate was determined by study of the respiratory data obtained during this time and by terminal analyses of the gastrointestinal tract and the blood for glucose and of the whole liver and a muscle sample (gastrocnemius) for glycogen. The details of these determinations and of the experimental procedures were the same as those used in similar studies on untreated rats reported previously (3).

Determinations of glycogen reserves and blood sugar levels, as well as of the B.M.R., were made also on unfed hypophysectomized rats previously treated with thyroxin.

In the tables presented below, the figures for approximate error have been calculated from the usual formula for probable error of the mean, $0.675 \frac{s}{\sqrt{n-1}}$.

Results. The results of these experiments are presented in the accompanying tables, together with comparative figures on normal rats, and on untreated hypophysectomized animals. From these figures it can be seen (table 1) that the thyroxin treatment effected complete restoration of the glucose absorption rate. The oxygen consumption was increased, although not to normal, by the doses of thyroxin given. In spite of these changes no significant differences were observed in the R.Q.s, or in the proportion of absorbed carbohydrate apparently oxidized. As would be expected, therefore, the total amount of carbohydrate oxidized was greater in the treated rats than in either normal or untreated hypophysectomized animals. The effect of the thyroxin on carbohydrate levels in the fed rats (table 2) was approximately proportional to the change in the absorption rate. The maintenance of carbohydrate in the fasted hypophysectomized rats was not improved in any case by the thyroxin treatment. From these data it appears that though thyroxin increases the intestinal absorption rate, it does not otherwise alter the effects of hypophysectomy on carbohydrate metabolism.

The effects of thyroxin treatment stand in contrast to those of A.P. extracts; for, when given in acute tests, the latter preparations promptly depress the R.Q. during glucose absorption, and increase the amount of carbohydrate found in blood and tissues (3). They do not cause any change in either the B.M.R. or glucose absorption rate under these conditions. However, thyrotropic extracts causing an increase in the B.M.R. when given over a period of several days would be expected to increase the glucose absorption rate at the same time, if the thyroid secretion is the active agent as indicated here.

The dose of thyroxin first given was 10 gamma daily. This small dose restored the absorption rate to normal in these cases, but it produced an

TABLE 1

*Metabolism of hypophysectomized rats fed glucose*

| | HYPOPHYSECTOMIZED RATS | | NORMAL RATS |
|---|---|---|---|
| | Untreated | Thyroxin treated | Untreated |
| 1. Number of rats | 10 | 9 | 10 |
| 2. Glucose absorbed (mgm./100 g./hr.) | 122 ±3 | 181 ±3 | 189 ±4 |
| 3. $O_2$ consumption (ccm./100 g./hr.) | 106 ±3 | 130 ±3 | 147 ±2 |
| 4. RQ | 0.908 ±0.007 | 0.919 ±0.006 | 0.857 ±0.005 |
| 5. Nitrogen excretion (mgm./100 g./hr.) | 2.2 | 2.2 | 1.9 |
| 6. RQ-(non-protein)* | 0.925 | 0.931 | 0.862 |
| 7. Carbohydrate oxidized in experimental period (about 4 hrs.)* | | | |
| a. mgm./100 g./hr. | 420 | 520 | 453 |
| b. Per cent of absorbed glucose | 74 ±3.7 | 67 ±1.7 | 53 ±1.5 |

* The average value for nitrogen excretion was used for these calculations in each case.

TABLE 2

*The effect of thyroxin on carbohydrate levels of hypophysectomized rats fasted and fed glucose*

| | NUMBER OF RATS | BLOOD GLUCOSE | LIVER GLYCOGEN | | MUSCLE GLYCOGEN |
|---|---|---|---|---|---|
| | | | Mgm./100 g. body weight | Per cent of absorbed glucose | |
| | | *mgm. per cent* | | | *mgm. per cent* |
| Hypophysectomized rats | | | | | |
| Untreated | | | | | |
| Fasted | 10 | 55 | 0.5 | | 350 |
| Fed glucose* | 10 | 77 | 25 | 5 | 520 |
| Thyroxin treated | | | | | |
| Fasted | 5 | 52 | 0.5 | | 312 |
| Fed glucose* | 9 | 109 | 40 | 5 | 559 |
| Normal rats | | | | | |
| Untreated | | | | | |
| Fasted | 15 | 72 | 1.2 | | 524 |
| Fed glucose* | 10 | 132 | 123 | 14 | 762 |

* Four hours after feeding.

increase of only 10 to 15 per cent in the oxygen consumption. The larger dose of 20 gamma was therefore used in the later experiments. Since even this dose did not always effect complete restoration of the metabolic

rate, it is evident that the effective dose for restoring the absorption rate in hypophysectomized rats is approximately half that required to affect the B.M.R. to the same degree under these conditions. It may be noted also that in the complete series of treated animals there was no correlation between the oxygen consumption and glucose absorption rate; but such data are inadequate for a conclusion as to the connection between these two effects of thyroxin.

Since in the series presented here the rate of oxygen consumption was on the average still below normal, it might be considered that a further increase in the B.M.R. would perhaps produce some change in the results. However, in a short series of four treated animals, in which the metabolic rate was completely normal, the other figures for R.Q., carbohydrate oxidation, and glycogen storage were in complete accord with those presented here. Moreover, it seems unlikely that a further increase in oxygen uptake would have a depressing effect on carbohydrate consumption; if anything, it should increase it.

## SUMMARY

Thyroxin substitution therapy in hypophysectomized rats can restore the rate of absorption of glucose from the intestine completely to normal. The dose of crystalline thyroxin necessary for this action is less than that required to restore the metabolic rate. This treatment does not improve the maintenance of carbohydrate stores during fasting, or change the proportionate disposition of absorbed glucose in hypophysectomized rats.

I wish to thank Dr. Theodore L. Althausen for suggesting this investigation.

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# A STUDY ON THE TEMPERATURE NECESSARY TO CAUSE DEATH IN FATIGUED NEURONS AS COMPARED WITH RESTING NEURONS

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Received for publication February 19, 1938

Latimer (1898) first showed that fatigued muscle entered heat rigor at a lower temperature than non-fatigued muscle. Shafer (1933) pointed out that this was due to the uptake of water by the fatigued muscle. Moreover, other treatments—bathing the muscle with alkaline Ringer's solutions of certain pH values, or with hypotonic Ringer's solution, or poisoning with monoiodoacetic acid—which caused the muscle protoplasm to imbibe water, somehow conditioned the cells to undergo heat rigor at a lower temperature just as in the case of fatigue. The extra water taken up by the cells was the important conditioning factor. He suggested that, in the same way, fatigued neurons might increase in size by taking up water and then show a lower death temperature than normal. Nothing was found in the literature on the question of whether fatigued neurons are killed at a lower temperature than normal resting neurons, but contradictory findings were noted on whether fatigued neurons are larger than in their resting condition (see p. 123, Shafer, 1933).

Histological comparison of the average size of fatigued and of non-fatigued neurons in the dorsal spinal ganglia of the eighth or ninth pairs of spinal nerves of the bullfrog was undertaken, but abandoned when it became clear that to establish the proof of possible but very small differences would require an impracticable amount of work. We turned, therefore, to an oscillographic study of the thermal death point in partly fatigued and in resting neurons—action potential (designated A.P., hereafter) being used as the criterion of the state of activity in a nerve when stimulated. This study would throw light on the question of enlargement of neurons during fatigue, by analogy from findings already quoted on muscle cells, if we could determine whether neurons die at a lower temperature when partly fatigued. For suitable study material in this connection, we decided on either the eighth or ninth pair of spinal nerves of the bullfrog because these could be removed with long dorsal and ventral roots for taking A.P.s (J. Erlanger, G. H. Bishop and H. S. Gasser, 1926). Action potentials were determined with an R.C.A. 905 oscillograph tube, em-

ploying a self-contained linear electron sweep circuit, for the time axis, synchronizable with the observed potentials. With a five-stage condenser-resistance, coupled amplifier the overall amplification was 100 microvolts = 16 mm. on the oscillograph tube. To achieve a low noise level and low grid current, the latter minimizing polarization to the lead off electrodes, a 38 tube was employed in the first amplifier stage in the manner described for the glass electrode electrometer (Skow and Wynd, 1936). Time intervals, when required, were obtained from a standardized beat frequency oscillator with a range of 10 sec. to 0.0001 sec., or from the 60 cycle source. In the early part of our work we tried a sine wave beat frequency oscillator for our stimulator, but soon discarded this (see Coppée, 1936) for a direct current neon tube oscillator[1] (Reschovsky and Scheminzky, 1935). The stimulation frequency of the latter could be easily and quickly checked with the standardized sine wave oscillator or by multiples of 60 cycles. Its circuit constants were so arranged that stimulation voltage and duration were adequate to overcome any slight electrode polarization or rise of nerve threshold. Its compactness facilitated almost complete balancing out of shock artifact at high amplification.

Before beginning an experiment, we tested the nerves for injury by observing whether the sensory and motor roots, respectively, of the pair gave reasonably comparable A.P.s. At a voltage amplification of 6000 the average sensory roots gave an A.P. curve about 32 mm. high; and the motor roots, 64 mm. high at the beginning of the experiment—and the apparatus gave very sharp clean curves. The stimulus was applied to the trunk of the nerve, so that motor and sensory fibers were stimulated together, and the A.P.s. could be taken from either nerve root.

The two excised nerves were borne side by side on silver electrodes set in a bakelite plate—two stimulating electrodes for each nerve trunk and two lead off electrodes for each nerve root. This electrode plate was rigidly suspended 12 cm. beneath a heavy cork by four brass tubes which passed through the cork and fastened into the plate at their lower ends. Multipolar switches were fastened to the upper surface of the cork and all wires connected with the electrodes were sealed moisture proof in the electrode plate, and in the brass tubes so that the lead off wires were shielded to the point where they connected with the switches. Thus the stimulus could be quickly changed from one nerve trunk to the other, and A.P. could be as easily taken from either root of either nerve. The electrode plate was contained in a small glass air chamber into which the heavy cork fitted and this chamber was surrounded with a water bath by which temperature changes were controlled. The nerve temperatures were taken from a thermometer inserted into the air chamber with its

[1] The neon tube stimulator and the C. R. oscillograph were constructed by R. K. Skow.

bulb resting upon the bakelite plate. This part of the apparatus could be easily disconnected from the stimulator and the oscillograph and carried to the dissecting table where the nerves were carefully put on the electrodes. The ganglion of each nerve rested in a shallow well of Ringer's solution in the bakelite plate. The part of each nerve trunk between this well and the proximal stimulating electrode lay upon squares of blotting paper soaked with Ringer's solution. The electrodes were forked at the ends and in each fork was placed a tiny wedge of blotting paper soaked in Ringer's. The nerves lay upon the soaked wedges, and another small wedge of soaked blotting paper was placed, between the forks, on top of the nerves. Thus, at each contact, there was a wet connection of Ringer's solution surrounding the nerve. Soaked filter paper lined the upper part of the electrode chamber. Also, a little water was placed in the lower part of the chamber beneath, but not touching, the bakelite plate. This insured against any drying of the nerves during the heating process. Resting nerves could be kept in perfect condition in the chamber at room temperature for twelve hours, which was much longer than our regular experiments lasted.

We carried on a great many experiments to try out the possibilities of the apparatus and to study the effect of changing various factors which enter into the experiments, or which we suspected might affect our results, before undertaking the series of experiments reported in this paper.

Because of the low metabolic rate of nerves and the rapidity of their recovery after stimulation (Hill, Downing and Gerard, 1926) and especially because the metabolic rate of active nerve as indicated by oxygen consumption, does not greatly exceed that of resting nerve (Schmitt, 1933; Schmitt and Gasser, 1933), it seemed desirable to fatigue the one nerve of a pair rapidly and to keep it in the fatigued condition while it and its resting mate were undergoing the heating process. Stimulation was continuous, therefore, at room temperature until A.P.s were reduced to about 12 per cent of their initial value. Then the heating process was begun, but stimulation was not stopped until the end of the heating process.

For the series reported in this paper, experiments were grouped at the following rates of stimulation, viz., 40, 120, 180, 240 stimuli per second—although later, five experiments were run at 300 stimuli per second, and two experiments at 360 stimuli per second. To prevent oxidative recovery and thus hasten the fatigue process, we tried some of the experiments in carbon dioxide. But pure carbon dioxide, as an atmosphere for the nerves, might introduce a disturbing factor through lowering the pH of the nerves more than did mere fatigue in air. We tried three experiments, therefore, in nitrogen containing 5 per cent, only, of carbon dioxide as a possible check. Fatigue periods (i.e., to 12 per cent of initial A.P.) in the absence of oxygen were always shorter than when carried out

in air, but, of course, there was the disadvantage that the resting nerve was also without oxygen. Some accumulation of products of catabolism in the resting nerve, with increase in osmotic pressure above what would have been the case in air, might be expected after a few hours, but scarcely so within the period of our experiments. The ideal situation was to keep the resting nerve as nearly as possible in the normal state and to bring the stimulated nerve to a severely fatigued state (but still showing a little A.P., with capacity for recovery) at such rate as to cause a maximal accumulation of waste products in the sensory neurons and the motor fibers, with little or no diffusion.

During our preliminary experiments we discovered that when nerves were heated to the point where A.P. was completely extinguished[2] (but were not heated much above that temperature) and then were cooled to approximately room temperature, they always recovered irritability again and gave more or less A.P. That is, the changes taking place in the heated nerve were still reversible in some degree *at* and even *slightly above* the temperature at which A.P. was lost. This was true of both the fatigued and the non-fatigued nerves. The A.P. extinction temperature did not prove to be the lethal temperature; it marked the temperature at which irritability was lost, merely. By heating to definite increments above the A.P. extinction temperature in different experiments, then cooling and again testing for possible recovery, we learned that the lethal temperature (i.e., that above which there was irreversible loss of A.P.) lay somewhere between 43° and 45.5°C. It varied a little with different nerves, apparently for reasons we could not control by any care we could use in dissection and manipulation. Since this problem was undertaken, a paper by Schmitt and Fourt (1936) on thermal inactivation has appeared in which they find complete extinction of action potential at 43.2°C. At that temperature their results show respiration was reduced only 10 per cent; above 44°C. it rapidly fell to zero. The latter temperature is near what we have shown to be the lethal temperature.

For the series which we report in this paper we proceeded as follows.

[2] By "extinction of A.P. (irritability)," we mean that the oscillograph record either showed no indication whatever of an A.P. curve (as was the usual case)—even when the strength of stimulus was doubled and the voltage amplification was increased to 300,000—or, that the height of the curve would be not greater than 0.2 mm. under the test (i.e., showing a "trace" of A.P.). Thus, by exploratory, preliminary experiments, and by checks made during our series, we made sure that the intensity of the stimulus was always greater than that necessary to give a maximal response—even under conditions of slight polarization or increase in threshold. Moreover, our stimulator had six condensers of different capacity (and duration of discharge) so that we could make sure that loss of A.P. was not the result of change in chronaxie. Therefore, when A.P. was absent during our stimulus, we believe that the nerve was non-irritable under its condition at that time.

We recorded the temperature at which irritability was lost, in each root of the nerve under fatigue stimulation, by observing the temperature at which A.P. was extinguished—and likewise in the roots of the resting nerve, using only as many stimuli on the trunk of this nerve as were necessary to measure height of A.P. in quick tests. Stimulation of the one nerve was continuous, at the particular rate chosen, except for the short intervals required for tests of A.P. in the resting nerve. At each temperature at which measurements were made, during the rise in temperature, the heights of A.P., in the roots of both nerves, were recorded until finally no A.P. appeared in any nerve root. Following this, the temperature was brought to a little higher value—the "highest temperature" for that particular experiment—and held at that value (within about 0.2°C.) for one minute. At this point, stimulation of the nerve under fatigue was stopped.[3] Then at once, the nerves were cooled rapidly (by changing the water bath) to nearly room temperature. The room temperature at which the experiments were carried on was about 20°C. The average temperature to which the nerves were cooled, before beginning to test for possible recovery, was 24°C. and the average time used in the heating process was forty minutes. After fifteen minutes, or a little longer, at the recovery temperature, examination tests were begun for possible recovery.

By the above plan if A.P. (irritability) were lost at a lower temperature in the fatigued than in the resting nerve, our readings would show that—and if the fatigued nerves really had a death temperature significantly lower than that of the resting mates, we could reasonably expect to find, in *some of our experiments*, that the resting nerves would show recovery of A.P. in either sensory or motor roots (or in both) and not in the corresponding roots of the fatigued mate.

In order to condense results and get them into more easily comparable form, averages for each group of experiments were calculated, and these have been incorporated in tables. In table 1 we have grouped all experiments carried out in air, all those carried out in carbon dioxide, and those in which the nerves were confined in nitrogen containing only 5 per cent of carbon dioxide, to show the average temperatures at which irritability was lost for fibers of the nerve trunks which pass into the dorsal roots and into the ventral roots of the fatigued nerves, as compared, respectively, with the average temperatures at which it was lost for fibers passing into the corresponding roots of the resting mates.

In air and in carbon dioxide the average temperature at which irritability

[3] It appeared to make no difference in ability to recover, whether we stopped stimulation as soon as irritability (A.P.) had been extinguished, or whether we continued stimulation until the highest temperature was reached. The nerve was unaffected by further stimulation after irritability was lost, as might be expected.

was lost was lower in each root of the fatigued nerve than in the corresponding root of the resting nerve, by the amounts shown in the two right-hand columns of the table. In each case these temperature differences between the sensory roots are a little greater than between the motor roots—the differences, in all cases, being greater for the experiments carried out in carbon dioxide. In case of the three experiments carried out in nitrogen plus 5 per cent carbon dioxide, the temperature at which irritability was lost in the roots of the resting nerves was not obtained, but a small A.P. was still present in them at 41°C. In the fatigued nerves of this group, however, irritability was lost in the two roots at temperatures a little more comparable with those of experiments carried out in air than with those in the pure carbon dioxide group. The average total fatigue period was shortest in carbon dioxide (52+ min.), intermediate in nitrogen plus 5 per cent carbon dioxide (85 min.), and longest in air (219 min.).

TABLE 1

*Temperature at which action potential (i.e., irritability) was lost*

| GAS USED AROUND THE NERVES | NUMBER OF EXPERIMENTS USED | AVERAGE HIGHEST TEMPERATURE USED | AVERAGE TEMPERATURE AT WHICH IRRITABILITY WAS LOST IN | | | | a–a′ | b–b′ |
|---|---|---|---|---|---|---|---|---|
| | | | Resting nerve | | Active nerve | | | |
| | | | Sensory root a | Motor root b | Sensory root a′ | Motor root b′ | | |
| | | °C. | °C. | °C. | °C. | °C. | °C. | °C. |
| Air | 16 | 44.1 | 42.5 | 42.2 | 41.4 | 41.5 | 1.1 | 0.7 |
| $CO_2$ | 10 | 41.8 | 39.8 | 39.8 | 36.7 | 37.8 | 3.1 | 2.0 |
| N 95 per cent + $CO_2$ 5 per cent | 3 | 44.5 | Not taken, but present at 41 | | 40.0 | 41.0 | | |

Since, as already indicated, there was usually some recovery of A.P. in the roots of both nerves of a pair after the process of fatigue and heating, the results were studied with reference to the average per cent recovery of A.P. in the sensory and motor roots of the fatigued nerves, as compared with the average per cent recovery in the corresponding roots of the resting mates of the pairs of nerves—A.P. being taken always under maximal stimulation. These averages, grouped for 40 stimuli per second and 120 stimuli per second in air, and for 180 stimuli per second and 240 stimuli per second in carbon dioxide, are given in table 2. The average per cent recovery of height of A.P. in the roots of the resting nerves is greater in every case than the average per cent recovery in the corresponding roots of the fatigued nerves. For three of the groups, column A shows the recovery of A.P. was five times (or more) greater in the resting sensory than in the fatigued sensory roots of the pairs of nerves. Column B

shows, in two of the groups, that the motor roots of the resting nerves gave a little less than five times greater recovery of A.P. than the motor roots of the fatigued nerves. In the case of the group of experiments with forty stimuli per second, we found at the end of one experiment, during the series, that moisture had crept between one of the A.P. electrodes for the right motor root and the bakelite, causing leak. That particular experiment was thrown out. The moisture was dried out and the leak corrected (using hard asphaltum) so that we had no more trouble. We know that no leak was present when the series was started but it may have developed to a *slight* degree causing some incorrect readings before we discovered it. If so, that might readily account for the low figure of 1.17 in column B. Why the recovery should be less than twice as great in the sensory and motor roots of the resting nerves than it was in the corresponding roots of the active nerves in case of the experiments

TABLE 2

*Recovery of action potential*

| STIMULI USED PER SECOND | GAS USED AROUND THE NERVES | NUMBER OF EXPERIMENTS USED | AVERAGE HIGHEST TEMPERATURE USED | AVERAGE PER CENT RECOVERY, AFTER FATIGUE AND HEATING, IN | | | | A a/a′ | B b/b′ |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Resting nerve | | Active nerve | | | |
| | | | | Sensory root a | Motor root b | Sensory root a′ | Motor root b′ | | |
| | | | °C. | *per cent* | *per cent* | *per cent* | *per cent* | | |
| 40 | Air | 7 | 43.3+ | 28.1 | 34.8 | 4.9 | 29.5 | 5.7 | 1.17 |
| 120 | Air | 6 | 44.9 | 6.0 | 20.0 | 1.2 | 4.1 | 5.0 | 4.9 |
| 180 | $CO_2$ | 3 | 43.0 | 30.4 | 42.9 | 20.9 | 34.5 | 1.4 | 1.2 |
| 240 | $CO_2$ | 5 | 40.7 | 29.1 | 33.6 | 5.4 | 6.9 | 5.3 | 4.8 |

with 180 stimuli per second in carbon dioxide, we do not know—but it was consistently so in all three experiments. For some reason, A.P.s of alternate stimuli were very uneven in these three experiments and that is why we stopped with only three experiments in the series. It seemed not to be a good rate of stimulation under our conditions. In spite of the fact that A.P. curves were uneven, however, fatigue was more rapid at this rate in carbon dioxide than at 120 stimuli per second in air—although not nearly so rapid as at 240 stimuli per second. At 300 and at 360 stimuli per second the alternate A.P. heights were quite irregular—the rate of stimulation being clearly too high after the first two minutes of stimulation. Averaging the values in "A" and in "B" for all experiments in table 2, we find that the resting sensory nerve roots showed 4.3 times better recovery of A.P. and the resting motor roots 3.0 times better recovery of A.P. than did the corresponding roots of the fatigued nerves (mates).

In a group of nine experiments carried out (as a check) with pairs of

nerves, neither of which was fatigued, the average temperature at which irritability was lost varied only 0.3°C. as between sensory and motor roots of the right and left nerves and the extreme range of variation in the different pairs of nerves in the nine experiments was 3.0°C. The average per cent recovery of A.P. in the right sensory roots, after heating both pairs of resting nerves until irritability was lost, then cooling to room temperature and testing (after fifteen minutes at room temperature) differed from that of the left sensory roots by only 1.5—i.e., the larger value was only 1.1+ times larger than the smaller value. The average per cent recovery of A.P. in the right motor roots differed from that of the left roots by 1.4—i.e., the larger was again only 1.1+ times greater than the smaller.

*We had only two experiments in which the resting nerve recovered A.P., in both roots, after heating, while the fatigued nerve showed no recovery of A.P.* In these two experiments the average recovery of A.P. height for the sensory and the motor roots of the resting nerves was only 0.7 per cent and 2.2 per cent respectively. Recovery being so slight, it seemed clear that the fatigued nerves were killed at a temperature not very much lower than the temperature which would have been fatal to the resting mates.

*We had no experiment in which the fatigued nerve showed recovery of A.P. in both roots while its resting mate did not.* There was one experiment, however, in which the sensory roots of both the active and the resting nerve (mates) were killed by heating to 45°C. while the motor roots of both nerves showed some recovery of A.P. after cooling. In other experiments than the above three, either both roots of the resting and of the fatigued nerves showed some recovery of A.P. (with less recovery in the fatigued nerves, as reported in table 2) or both were killed by the highest temperature used in the experiment. We had seven experiments in this last category—i.e., in which the nerve fibers of both roots of both nerves were killed by the highest temperature to which they had been subjected for one minute. In these seven experiments, the average "highest temperature" used was 44.7°C. (variation between extremes, 2.5°C.). In case of the sensory roots of the *resting* nerves, irritability (judged by extinction of A.P.) was lost at an average of 2.3°C.—in the sensory roots of the fatigued nerves, 3.6°C.—below the average highest temperature used. Motor roots of resting nerves lost irritability at 2.5°C., and motor roots of fatigued nerves at 3.7°C. below the average highest temperature (44.7°C.) used in this category. In case of the two experiments in which both roots of the resting nerves recovered A.P. while neither root of the fatigued nerves recovered, the irritability was lost in the fatigued nerves at a much lower temperature—average 6.5°C. lower for the sensory and 5.5°C. lower (average) for the motor roots—than the 44°C. average which had proven to be a lethal temperature for the fatigued nerves but not for their resting

mates. Our results indicate that the same factor (probably uptake of water) which caused the fatigued nerves to lose irritability at a lower temperature (and to show less recovery ability of A.P.) than its resting mate, caused it to be subject to a lower death temperature than its resting mate, also; but the death temperature difference was much smaller than the difference between temperatures at which the fatigued and the resting nerves lost irritability. Only when the latter difference was marked, was the death temperature difference a very appreciable amount, apparently. On that account, therefore, one was not likely to guess, correctly, what "highest temperature" would prove to be a killing temperature for the fatigued nerve but not for the resting mate, unless (as in the two cases in which we did guess correctly) the difference was large between the temperatures at which irritability ceased in the two nerves. Then the death-temperature for the fatigued nerve fibers and that for the resting fibers became separated widely enough so that we were more likely to make a successful guess and place our "highest temperature" for the experiment between these two death temperatures. If, instead of being compelled to make approximations of the death temperature in every case, we could have determined it with the accuracy with which we could determine the temperature at which irritability was lost, our problem would have been less difficult. However, Professor Weymouth of this Department has treated the various factors considered in our experiments by Fisher's method of analysis of variance (Fisher, 1935) and has found our results to be significant. There is less than two chances in one hundred that a difference in recovery between resting and stimulated nerves of the magnitude reported could have arisen by chance. The ratio of the variance due to activity to that due to error (F) is 13.223 while the "1 per cent" point for these degrees of freedom is 6.92.

We performed one experiment in which the pair of nerves was cooled to 3°C. for the fatiguing process. Certain details of this experiment should be reported for reasons that will be evident. The active nerve was given 120 stimuli per second for 120 minutes, and at the end of that time the height of the A.P. of the sensory neurons had been reduced 94.5 per cent; that of the motor fibers 96.6 per cent. Irritability was lost in both the sensory and motor roots of this nerve at 43°C. in air. The nerve chamber was brought up to 45°C. for one minute, then cooled rapidly to 20°C.; and after 18 minutes both the sensory and the motor roots were tested for recovery. The sensory showed no recovery and the motor showed a barely discernible recovery (i.e., A.P. was about 0.1 mm. or less). Thus the behavior of the nerve with respect to the temperature at which irritability was lost (and with respect to the death temperature) was not different from nerves fatigued at a room temperature of 20°C.; although, as the nerve warmed up in the process of heating the chamber,

the A.P. at first improved as the temperature increased, until at 31.5°C. it was higher than at 4°C. before the fatiguing process began (sensory 14 mm., motor 52 mm. at 31.5°C. as against sensory 9 mm., motor 29 mm. at 4°C.). As the rising temperature reached the optimum for the *resting* mate, it showed a far more marked increase in height of A.P.s—that of the sensory resting neurons increased to 400 per cent of the value at 4°C. (56 mm. compared to 14 mm.); that of the resting motor fibers went off the scale and could not be read at the amplification we were using. As the temperature continued to increase after that, the A.P.s declined, at first slowly, then more rapidly, until at 43°C. all irritability was lost and no A.P. could be detected in either root of the active nerve; irritability was lost in the resting sensory fibers at 44°C.—in the resting motor fibers at 45°C. At 40°C., however, the A.P. of the active nerve was giving sensory 6 mm., motor 28 mm., although it was extinguished entirely, just three minutes later, at only 3°C. higher temperature. The sensory fibers of the resting nerve, at 40°C., gave an A.P. curve 20 mm. high; and the motor, 66 mm. high—although these were also extinguished soon afterward with a further rise of only 4°C. and 5°C. respectively. In other words, as the critical temperature for fibers in each root of the nerves was approached, A.P. fell off more and more rapidly. This was generally true in all the experiments and it shows that, *for the time intervals we used in the heating process*, the degree of temperature attained was the important factor affecting the irritability of the nerve fibers—rather than the time interval. This particular experiment, in which we carried out the principal part of the fatiguing process at such a low temperature, served to emphasize that point. In this experiment, also, we measured the velocity of the nerve impulses producing the main A.P. of the sensory fibers at 4°C. (5.68 meters per second), at 3°C. (3.57 meters per sec.), and again, after the fatiguing process, at 40°C. (32.15 meters per sec.). In another experiment, we measured the velocity of the nerve impulse in the sensory fibers of the fatigued nerve that had been heated to 45°C., after recovery-cooling for 15 minutes had brought back irritability with a barely discernible A.P. The measurement made at 25°C. gave a velocity of 19.32 meters per second. It must have been the A group of fibers, therefore, that gave the slight A.P. on recovery (after being heated to near the lethal temperature), and that were the last to lose irritability at the end of a fatigue period during the heating process (see Erlanger and Gasser, 1930, 1937). We knew that we were measuring the height of the A.P. of the large, or A, group of fibers at the beginning of an experiment, but after fatigue (or after recovery from heating) we had not been certain that we were dealing with the A.P. of the same fibers. The velocities of the nerve impulses, however, seem to make it clear that it was the fibers of the A group that withstood the ordeal of fatigue and heating best.

We then tried and found that we could take a pair of non-fatigued nerves, place one in Ringer's solution in our temperature-stimulating chamber and the other in hypotonic Ringer's solution (1 cc. Ringer's diluted to 5 cc. with water) in the same chamber, and prove, by testing, that the A.P. in the latter became reduced lower and lower until, if time enough were given, it became extinguished entirely at room temperature. After the one nerve had been treated in hypotonic Ringer's until its irritability was lost at a low temperature when the chamber was heated—and the heating of both nerves had been continued further until the mate (in Ringer's) had just lost irritability—one could then replace the hypotonic solution with Ringer's solution around the treated nerve, and find by testing that the treated nerve could not recover after the heating process while the mate could recover 12 per cent or more of its A.P. That is, uptake of water from hypotonic solution lowers the resistance of the neuron and the nerve fiber to heat just as it does in case of the muscle cell.

A comparison of results from sensory roots and motor roots in these experiments indicates that the presence of the sensory cell bodies made the sensory fibers a little more subject to the effect of heat, after fatigue, than were the motor fibers (with no cell body included), but the difference is not great. The effect of fatigue in lowering the critical temperature at which damage or death results is a recognizable effect by the method we used but the lowering of the critical temperature is slight as compared with the similar effect in the case of muscle. This is probably to be explained by the fact that increased metabolism in the nerve during activity is relatively slight when compared to the increased metabolism of active muscle over that of resting muscle. The increase in osmotic pressure and the uptake of water of the active neuron or nerve fiber would therefore probably be very slight as compared to the increased osmotic pressure and the uptake of water in rapidly fatigued muscle cells. Our results would indicate by analogy that any increase in size of neurons due to fatigue by stimulation such as we used must be very slight, indeed, and would be hard to detect with certainty by present histological methods.

## SUMMARY

1. Sensory neurons and motor nerve fibers which have been fatigued rapidly by maximal stimuli at 40, 120, 180 or 240 shocks per second lose their irritability (A.P.), when heated, at an average temperature slightly lower than do their resting mates.

2. The temperature required to cause death is slightly higher than that which causes loss of irritability in case of both the fatigued and the resting neurons and fibers.

3. Fatigued nerves are killed by a temperature only very slightly lower than that required for resting mates.

4. When fatigued and resting neurons and nerve fibers were heated to a temperature at which all irritability (A.P.) was just completely extinguished, they all recovered on cooling again, but the resting sensory neurons recovered an A.P. averaging 4.3 times greater than that of the previously fatigued mates; the resting motor fibers, 3.0 times higher than their fatigued mates.

5. The exact differences between resting and fatigued neurons, though not large in any case (being *very small* by comparison with results from muscle), are shown to be significant by Fisher's method of analysis of variance.

6. Considering that the greatly lowered heat-rigor temperature of fatigued muscle cells is associated with an uptake of water through the increased osmotic pressure resulting from their decidedly heightened metabolism during the fatiguing process, then by analogy, our finding of a *slightly* lowered death temperature in fatigued sensory neurons and motor nerve fibers would harmonize with their known comparatively *slight* increase in metabolism when active, and with an expected *slight* uptake of water—i.e., because little accumulation of waste products in the nerve cell body or the nerve fiber could be expected to occur, and hence any increase in osmotic pressure must necessarily be slight.

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# THE INFLUENCE OF THE UPRIGHT POSTURE ON THE METABOLIC RATE[1]

## With a Note on Standards

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Received for publication January 10, 1938

The maintenance of the upright stance without support is associated with an incessant shifting of the center of weight (Hellebrandt, 1938). A plumb passing through its mean position closely approximates in location the geometric center of the base (Hellebrandt et al., 1937) and thus brings about what Morton (1935) calls "a forwardly unbalanced position of the leg." The line of gravity, falling successively at various points in front of the ankle joint, represents a force which acts as a constantly changing stimulus for the geotonic contraction of the anti-gravity muscles. We have called standing *movement upon a stationary base*. This implies the liberation of energy for the performance of work. Although it is believed that the postural contraction of muscle occasions little augmentation of the calorific output (Roaf, 1912; Dusser de Barenne and Burger, 1924; Evans, 1930; Fulton, 1926; Weatherhead, 1932; Gaylor and Wishart, 1933), a concept of human standing as a dynamic phenomenon affected by mechanical factors in build warrants further study of the energy cost of standing in man. The object of these experiments was to determine the range of metabolic increase attributable to the brief maintenance of a passively assumed upright stance.

Methods. Total metabolism was determined in the post-absorptive state by indirect calorimetry, using the closed circuit type of apparatus. All observations were preceded by a half hour of rest in recumbency after taking additional precautions to avoid exercise in the interim between arising in the morning and the performance of the experiments. Basal determinations were made with the subject lying upon a comfortably padded tilting table. The upright posture was assumed without muscular effort. The feet rested against a footplate so constructed that it might be disengaged from the remainder of the apparatus, when the table was brought to the vertical, leaving the subject standing upon a low platform entirely without support. Standing metabolism was determined in a

[1] Supported in part by a grant from the Wisconsin Alumni Research Foundation.

stance as comfortable and natural as was compatible with the limitations of the equipment which necessitated the partial support of the mouthpiece and valves by the subject, constraining slightly the poise of the head. Two to three minutes elapsed between the recumbent and vertical determinations, a time sufficient for the circulatory (Wald, Guernsey and Scott, 1937) and respiratory adjustments to the change in posture (Main, 1937). Single five minute determinations were made under standard conditions in the two postures being studied. Total metabolism was calculated in the usual way from the oxygen consumed, assuming an R.Q. of 0.82. The subjects were 75 professional students in physical education. They formed a homogeneous group of post-pubescent girls and young adult women of superior physical fitness. Many were already familiar with the technique of the procedure, the personnel administering the tests and the surroundings in which the studies were conducted.

RESULTS. 1. *Basal values.* Because of the residence of the subjects in a region of simple endemic goiter, the unusual physical activity of their profession, and the fact that the majority were at an age of increased needs of the body for thyroxine, the basal metabolic values were carefully studied with a view to eliminating all who might be considered abnormal. This led to an incidental observation of sufficient importance to warrant its inclusion in this study.

The frequency distribution of the basal metabolic rate estimated by the Wisconsin Standard (Stark, 1936) is illustrated in figure 1. Although roughly normal, the mode is well to the *right* of the zero point and 29.33 per cent of this group of apparently healthy girls *exceed* the limits of the clinical norm which is usually set at $\pm 10$ per cent, being classified as abnormal were they pathologic, or, did the conditions under which the determinations were made yield a high incidence of hyperthyroidism. Metabolic rates are notoriously labile, and the influence of the vertical stance upon energy metabolism cannot be studied without first establishing the normalcy of the basal recumbent values with which the postural change is to be compared. An alternative explanation was that neither group nor procedure were open to criticism, but that the standard used was inapplicable to the subjects under study. The normal character of the frequency distribution curve gave this hypothesis credence. Basal rates were therefore recalculated by the Mayo Foundation Standard (Boothby, Berkson and Dunn, 1936). As illustrated in figure 1, the mode now falls to the *left* of the zero point and 22.66 per cent of this group of healthy girls is classifiable as *below* the clinical norm. The mean of the percentage deviations of the experimental from the two predicted values was therefore taken as most representative of the basal metabolism and those falling within $\pm 10$ per cent of this arbitrary zero were considered normal. The mean basal metabolic rate by the Wisconsin Standard was

+5.95 per cent, by the Mayo Foundation Standard −3.73 per cent and by the average standard +1.09 per cent.

2. *Standing metabolism.* The energy cost of standing was obtained by subtracting the basal maintenance rate from the total calorific output,

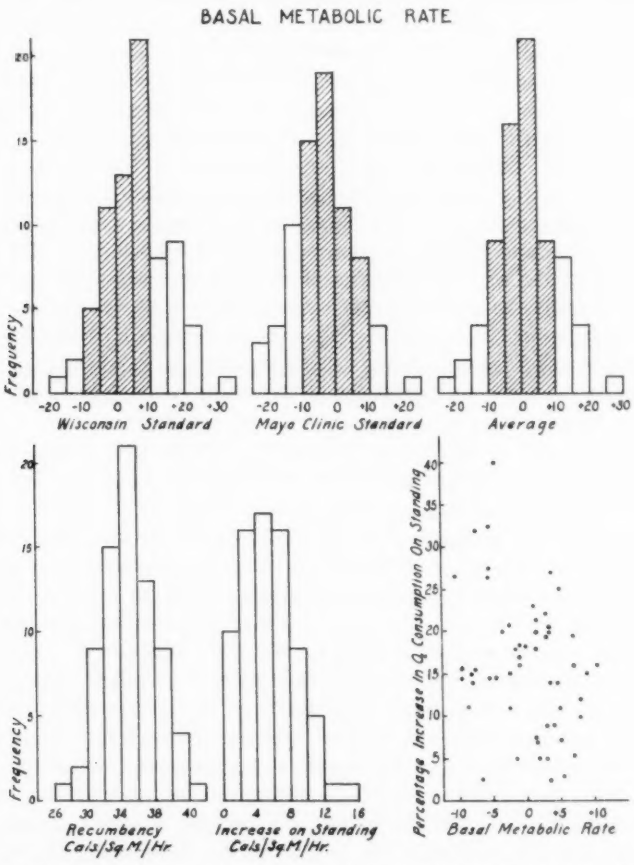


Fig. 1. Histograms showing the relation of the central tendency of the frequency distribution to the basal metabolism estimated by different standards, the frequency distribution of the energy metabolism in recumbency and standing, and the relation of the basal metabolic rate to the percentage increase in oxygen consumption with change in posture.

the mean values being respectively 35.12 ±0.248 cals./sq.m./hr. with a standard deviation of ±3.19 and 40.83 ±0.287 cals./sq.m./hr. with a standard deviation of ±3.69. This amounts to an increase of 5.71

cals./sq.m./hr. or 16.25 per cent. Casting out as abnormal all subjects with a basal rate exceeding ±10 per cent, leaves a group of 57 with a mean B.M.R. of +0.21 and an average energy requirement for recumbency and standing of 34.81 ±0.186 and 40.45 ±0.299 cals./sq.m./hr. respectively. The mean increase of 5.64 cals./sq.m./hr., an augmentation of 16.20 per cent, is almost identical with the mean values for the total group including those with basal metabolic rates above and below the clinical norm. This reëmphasizes the normal character of the frequency distribution of our data. Because the subjects were more fit than average it also suggests that although the chances are statistically great that those at the extremes of the distribution curve are abnormal, it does not necessarily hold that this applies to all falling beyond the customary clinical limits. Graphic representations of the energy estimations in calories per square meter per hour and the increase on standing are included in the illustration.

The only data in the literature strictly comparable to ours are those of Turner (1930) who performed 31 experiments on 12 subjects and found an average rise of 19 per cent in oxygen consumption on passively assumed standing. Our increases ranged from 1.9 to 88.1 cc. of oxygen per minute, the relative values varying from 0.90 to 50 per cent. There was no correlation between the basal metabolic rate and the increase in oxygen consumption on standing (fig. 1). Smith (1922) also found that the energy required for standing varied decidedly in different subjects, the mean value being an augmentation of 12 per cent. Katzenstein's (1891) values ranged from increases of 1.2 to 22 per cent. Douglas, Haldane, Henderson and Schneider (1913) report the highest mean value for relaxed standing, an increase in oxygen consumption of 38 per cent.

Discussion. The assumption of an identical respiratory quotient in recumbency and standing introduces an error difficult to evaluate. It has long been known that the determination of the R.Q. in short time experiments like those comprising this series is without value because of the blowing off of carbon dioxide. Pulmonary ventilation is augmented by the upright posture and the carbon dioxide content of the alveolar air falls. Main (1937) attributes these changes to reflex overstimulation of the respiratory center and postulates a relation between its functional state and the magnitude of the proprioceptive bombardment emanating from the muscles used in the maintenance of unsupported sitting and standing postures in man. In 1932 Harrison, Calhoun and Harrison demonstrated by direct observation on experimental animals in which only the sciatic nerves and blood vessels connected the amputated appendages with the remainder of the body, that afferent impulses from the muscles of the passively moved limbs stimulate the respiratory center. Higgins (1914) had already observed an inverse relation between muscular relaxation and alveolar carbon dioxide tension. All determinations of

energy metabolism made by indirect calorimetry other than those in which there is absolute muscular repose, are probably open to criticism unless any change in R.Q. due to alterations in energy metabolism can be differentiated from reflex influences upon the respiratory center. In short time experiments involving as mild an exercise as passively assumed comfortable standing, there is no reason to suspect a change in fuel from that metabolized at rest.

No attempt was made to gauge the steadiness of the stance. The subjects were carefully instructed to assume a natural and comfortable posture. Benedict and Murschhauser (1915) obtained fair uniformity in their standing metabolic values on repeated trials and inferred from these that the muscular contraction incidental to balancing and maintaining the body upright was relatively constant. We have shown that orientation with respect to gravity is also highly constant (Hellebrandt et al., 1937). The movements incidental to the mechanical instability of the upright stance are so accurately balanced that the average center of weight always falls close to the geometric center of the base. Although it may be assumed that the instability of normal standing subjects is also relatively constant because it is primarily the consequence of more or less fixed mechanical factors such as the height of the center of gravity, the size of the base of support and the magnitude of the gravitational stresses incidental to habitual postural attitudes, it must not be forgotten that postural contraction is also reflexly affected by innumerable stimuli from exteroceptive receptors and that tonus is susceptible to alteration as a result of hydrostatic ischemia of the brain. The multiplicity of the factors capable of modifying the degree of postural contraction and the stability of the stance may account for the wide variance in the energy cost of the standing of different subjects. The work of Morton (1935) suggests that shifts of the center of weight within certain relatively wide limits may affect the cost of standing little since the rotational stresses are being met by postural contraction alone, whereas disequilibrium of a degree which throws the projected center of weight beyond a series of critical points in the supporting base, calls forth the more costly phasic contraction of muscle. As yet we know neither the relation of the steadiness of a stance to the degree of equilibrating contraction necessary for its preservation nor the point at which postural contractions merge into phasic contractions for the maintenance of the vertical stance.

## SUMMARY

Total metabolism was determined by indirect calorimetry in 75 women in recumbency and passively assumed standing. The average increase on standing was 5.71 cals./sq.m./hr., or 16.25 per cent, varying markedly from subject to subject. The oxygen consumption of the 57 subjects

with a basal metabolic rate ±10 per cent showed a mean rise of 16.20 per cent. This amounts to 5.64 cals./sq.m./hr. A comparison is made of significant differences in the levels of standards and the interpretation of the clinical norm is discussed. A relation is postulated between the energy cost of standing and the type of contraction necessary for the preservation of the stance.

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# SOME OBSERVATIONS ON THE EFFECT OF EXERCISE ON THE BLOOD, LYMPH AND MUSCLE IN ITS RELATION TO MUSCLE SORENESS

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Received for publication August 2, 1937

Muscle soreness as the result of exercise may be divided into a number of types. First, due to trauma in which the pain comes on immediately. Second, the pain described by Hough (1902) and later Lewis (1925, 1932), Kissen (1934) and Perlow, Markle and Katz (1934). This type is produced probably by the accumulation of metabolic products or by anoxemia or ischemia. The pain comes on during the exercise and becomes so intense that the exercise must be stopped. Recovery is said to occur in about ten minutes. Third, a type of soreness that comes on some time after the exercise, usually next day, in which the muscles feel stiff and sore. This type of soreness most everyone has experienced as a result of unaccustomed exercise (such as walking, playing golf, etc.). This latter type is the soreness with which this paper deals.

That there should be these delayed changes in muscle, is rather peculiar since the work of Hill, Long and Lupton (1924) has shown, by measurements of heat production and oxygen consumption, that the muscles have returned to normal about an hour after the exercise. It is generally thought that this type of soreness and stiffness has something to do with the blood supply, lymph production or osmotic properties of the muscle. It was to test this hypothesis that these experiments were carried out. Because of the difficulties involved in satisfactorily measuring the amount of muscle soreness, it is necessary that the subject be approached with as purely physiological methods as possible. For this reason the methods involved the study of the blood, lymph, and muscles.

In our own work the problem has been attacked along three lines: 1, the problem of blood and its changes with exercise, especially with water content. Supplementing this part are some observations of the relation of the spleen to blood changes in exercise; 2, the changes in muscle weights and water content as the result of exercise; 3, the production of muscle soreness in the human subject and the measurement of any volume changes occurring in the muscle as a result of exercise, with observations during the period of muscle soreness and recovery.

METHODS AND PROCEDURES FOR THE DETERMINATION OF WATER CONTENT OF BLOOD IN NORMAL AND SPLENECTOMIZED DOGS AS THE RESULT OF EXERCISE. Exercise produces a concentration of the blood as has been shown by many observers. Probably Hawk (1904) studied the greatest variety of exercises and Dill, Talbott and Edwards (1930) have made the most extensive studies from the chemical side. Schneider and Crampton (1935) find no alteration for light work but a concentration for hard work. References to the work of others will be found in those papers. Concentration of the blood in the above experiments was judged either by an increase of red cell count or by hemoglobin determinations. Such an increase might either mean the addition of corpuscles to the blood or the abstraction of fluid from the plasma or a combination of both processes. Since the spleen is the only place known to have a reservoir of red cells and also to contract with exercise, experiments were first made to determine the effect of the spleen since most of the aforegoing mentioned experiments were made before the activity of the spleen was known.

For this purpose two normal dogs and two splenectomized dogs were trained to lie quietly, and to run on a motor driven treadmill. Following the training period a study was made of the water content of their blood before and immediately after exercise. Blood was drawn from the femoral artery and divided into four samples.

1. Put into a heparin tube for the determination of the relative quantities of corpuscles and plasma by the hematocrit method described by Bellis and Scott (1935).

2. Mixed with dry potassium oxalate for hemoglobin determination by an acid hematin method described by Scott (1917).

3. Put into a weighing bottle for determination of the water content by drying in a constant temperature oven at 90°C. to constant weight.

4. Put into a stoppered centrifuge tube and allowed to coagulate. It was then centrifuged and the serum pipetted off and put into a weighing bottle to determine its water content by the drying method.

The water content of the corpuscles was determined by calculation from the specific gravity of the whole blood, serum and corpuscles (Hammerschlag method, using a chloroform and benzol mixture), the water content of serum and whole blood and the hematocrit.

Each dog was given exercise on the treadmill for a period of five minutes, then for a period of ten minutes, etc., each period adding five minutes up to thirty minutes. The dogs were allowed at least two weeks' rest in their cages between exercise periods. Table 1 gives the results and averages of each dog for the whole experiment.

The changes produced in the blood by exercise do not seem to be related proportionally to the amount of exercise although several authors have

TABLE 1

| DOG NUMBER | SEX | DURATION OF EXERCISE | HEMOGLOBIN | | HEMATOCRIT PER CENT OF CORPUSCLES | | WATER CONTENT | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Serum | | Corpuscles | | Whole blood | |
| | | | Before exercise | After exercise | Before exercise | After exercise | Before exercise | After exercise | Before exercise | After exercise | Before exercise | After exercise |
| | | *minutes* | *per cent* | *per cent* | | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 1 | ♂ | 5 | 100 | 111 | 53.9 | 53.8 | 92.0 | 91.9 | 63.9 | 60.1 | 76.9 | 74.7 |
| | | 10 | 100 | 102 | 55.3 | 55.0 | 91.5 | 91.4 | 60.3 | 60.4 | 76.7 | 76.2 |
| | | 15 | 100 | 102 | 55.4 | 55.8 | | | | | 77.3 | 77.5 |
| | | 20 | 100 | 99 | 54.8 | 57.5 | 91.7 | 91.4 | 64.5 | 64.1 | 76.8 | 75.9 |
| | | 25 | 100 | 105 | 49.7 | 52.0 | | | | | | |
| | | 30 | 100 | 107 | 52.5 | | 91.2 | 90.6 | 63.3 | | 76.5 | 75.1 |
| Averages..... | | | 100 | 104 | 53.6 | 54.8 | 91.6 | 91.3 | 63.0 | 61.5 | 76.8 | 75.9 |
| 10 | ♂ | 5 | 100 | 110 | 48.5 | 50.9 | 93.1 | 93.1 | 67.0 | 66.5 | 80.2 | 79.4 |
| | | 10 | 100 | 107 | 48.0 | 53.4 | 92.6 | 92.3 | 65.2 | 64.7 | 79.2 | 77.6 |
| | | 15 | 100 | 110 | 48.5 | 52.4 | 92.8 | 92.6 | 65.2 | 64.9 | 79.1 | 78.0 |
| | | 20 | 100 | 136 | 46.0 | 57.2 | 92.1 | 91.4 | 64.8 | 63.4 | 79.2 | 75.5 |
| | | 25 | 100 | 110 | 49.3 | 54.6 | 92.9 | 92.2 | 65.4 | 64.4 | 79.2 | 77.1 |
| | | 30 | 100 | 117 | 47.6 | 55.9 | 92.3 | 91.4 | 65.1 | 63.8 | 79.0 | 76.0 |
| Averages..... | | | 100 | 115 | 48.0 | 54.1 | 92.6 | 92.2 | 65.5 | 64.6 | 79.3 | 77.3 |
| 6 | ♀ | 5 | 100 | 95 | 45.9 | 44.7 | 93.0 | 93.1 | 64.6 | 65.0 | 79.5 | 80.1 |
| | | 10 | 100 | 86 | 47.8 | 45.9 | 92.7 | 93.1 | 65.8 | 66.1 | 79.6 | 80.3 |
| | | 15 | 100 | | 38.5 | 37.3 | 93.6 | 93.4 | 66.8 | 67.3 | 82.6 | 82.8 |
| | | 20 | 100 | 94 | 31.9 | 30.5 | 93.2 | 93.2 | 68.0 | 68.8 | 84.1 | 84.6 |
| | | 25 | 100 | 85 | 40.9 | 38.8 | 92.6 | 92.6 | 66.7 | 66.2 | 81.4 | 81.5 |
| | | 30 | 100 | 92 | 41.6 | 40.3 | | 92.6 | | 66.4 | 81.2 | 81.3 |
| Averages..... | | | 100 | 90 | 41.4 | 39.6 | 93.0 | 93.0 | 66.4 | 66.6 | 81.4 | 81.8 |
| 9 | ♂ | 5 | 100 | 123 | 37.7 | 37.7 | 92.3 | 92.2 | 65.0 | 67.1 | 81.3 | 81.9 |
| | | 10 | 100 | 111 | 40.6 | 39.2 | 92.0 | 91.2 | 67.9 | 69.9 | 81.6 | 81.6 |
| | | 15 | 100 | 108 | 40.2 | 40.6 | 92.0 | | 67.6 | | 81.6 | 81.1 |
| | | 20 | 100 | 97 | 43.8 | 42.9 | 92.5 | 92.1 | 66.5 | 64.9 | 80.7 | 79.9 |
| | | 25 | 100 | 102 | 40.0 | 41.2 | 92.2 | 91.8 | 66.3 | 65.9 | 81.2 | 80.5 |
| | | | | 102 | 40.1 | 41.7 | 92.2 | 91.8 | 65.9 | 65.9 | 81.1 | 80.5 |
| | | 30 | 100 | 110 | 38.1 | 39.5 | 92.5 | 92.2 | 65.8 | 66.6 | 81.6 | 81.4 |
| Averages..... | | | 100 | 108 | 40.1 | 40.4 | 92.2 | 91.9 | 66.4 | 66.7 | 81.3 | 81.0 |

Numbers 1 and 10 normals.
Numbers 6 and 9 splenectomized.

claimed that there is a relationship according to the intensity. This latter phase could not be varied in this experiment because of the type of treadmill used.

The above experiments show that the spleen has little effect on the concentration of the blood in exercise. Dill, Talbott and Edwards (1930) also found that the blood of splenectomized men showed practically as great an increase in concentration as normal men doing the same work. The lone case of dog 6, is the one which arouses interest. The fact that her blood became diluted may be due to taking up of water from other tissues or from the loss of corpuscles from the blood stream. The latter explanation hardly seems plausible in a healthy animal even in the absence of the spleen. Barbour (1921) states that an increase in body temperature during exercise may produce a dilution of the blood. However, the temperature recordings in this dog were the same as in the normals.

*The effect of exercise on the water content of frogs' muscle.* Ranke (1865) in his classical work on tetanus showed that muscles gained in water content during exercise. This work has been corroborated since but in no instance is there mention of how long that water remained in the muscles.

It was the purpose of this experiment to see if the imbibed water was lost again and to get some idea of the time relationships.

The frogs (Rana pipiens) were anesthetized with ether and the sciatics both exposed. One nerve was stimulated once per second with single break shocks of submaximal intensity until there were no further visible signs of contraction in the gastrocnemius. In one frog the right sciatic was stimulated while in the next frog the left sciatic was stimulated. Sometimes one or both nerves were sectioned but we did not find this had any significance in the uptake of water. In each case a control frog was anesthetized and the nerves exposed but not stimulated. These control frogs were used to determine the normal difference in water content of the two gastrocnemius muscles in the same frog. All the muscles were dried to a constant weight at 90°.

The average water content of resting frog's gastrocnemius was 79.2 per cent (50 samples) while the muscles taken immediately after stimulation averaged 82.8 per cent, i.e., 3.6 per cent more (30 samples). In the control animals the difference in water content of the two muscles from the same animal varied from nothing to 1.3 per cent with an average of 0.5 per cent (50 experiments).

In order to determine how long the water was retained by the muscle the stimulated frog and its control were allowed to remain for a given time after the stimulation before exercising the muscles. In this group of experiments the nerve to the unstimulated muscle was cut. The loss of water is shown in the chart in which the difference in water content of the stimulated and unstimulated muscle is plotted against time. Each point on the curve is the average of two different experiments. We have no observations between 5 and 24 hours but at 24 hours the difference in water content was within normal limits.

When muscle contracts undoubtedly its osmotic pressure rises. Hill and Kupalov (1930) showed by measurements of vapor pressure that the osmotic pressure of resting frog's muscle was equivalent to 0.725 per cent NaCl while fatigued muscle was equivalent to 1.075 per cent. It is noted that the increase of water content is four to six times the normal variation. It also seems that muscles hold some of this water for a period between 5 and 24 hours. It is possible that the water is in the lymphatics and that it drains away very slowly with inactivity.

*Changes in muscle weights and water content as the result of exercise.* Rabbits were used in this experiment. In addition to observing the water changes in the muscle there was an attempt to determine the effects of exercise on the hemoglobin content in normal, splenectomized, acute

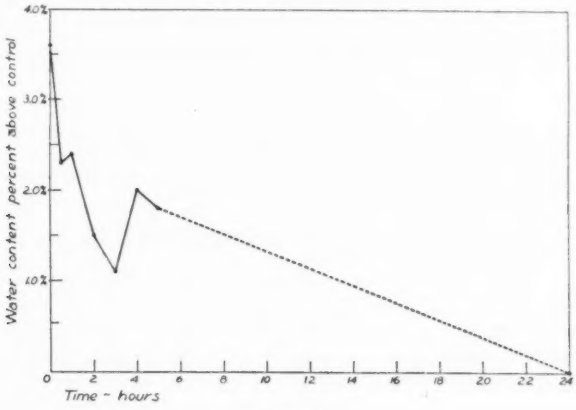


Chart 1

splenectomized (that is, splenectomized just before the animal was exercised) and in rabbits from which only one-half of the spleen had been removed. The spleens were removed by aseptic surgery and the animals allowed to recover for four to five weeks before being exercised.

This experiment was carried out in the following manner: A sample of ½ cc. of blood was taken from the median ear vein for hemoglobin determination. Following this the animal was weighed and given 30 mgm. of nembutal per kilogram of body weight intraperitoneally. When the anesthetic had reached its maximum effect, another sample of blood was taken from the median ear vein for two more hemoglobin determinations. The carotid artery was then exposed and a sample of blood (½ cc.) taken for hemoglobin.

Both sciatics were then exposed. In some cases the sciatic which supplied the muscle not exercised was sectioned, in others it was ligated

and in the remaining cases it was merely exposed. Electrodes were placed under the sciatic nerve of the muscle to be exercised. A sample of the muscle tissue (lateral belly of gastrocnemius) was removed from the leg which was not stimulated for determination of the water content. Following the excision, the muscle was carefully covered and the skin clipped to prevent drying of the tissues.

The muscle of the opposite leg was then stimulated to activity through its nerve. The stimulation was given three times every two seconds for a period of forty-five minutes. This was not sufficient to obtain signs of fatigue but muscle soreness does not necessarily require fatigue for its symptoms.

Immediately after the stimulation period another sample of blood was taken from the ear vein and one also from the carotid for hemoglobin determination. As soon as the blood samples were drawn, a sample of the muscle tissue was excised from each lateral gastrocnemius (stimulated

TABLE 2

*Hemoglobin determinations in per cent. First sample 100 per cent*

| RABBITS (AVERAGE OF) | MEDIAN EAR VEIN BLOOD | | | CAROTID ARTERY BLOOD | |
|---|---|---|---|---|---|
| | Before anesthesia | After anesthesia | After exercise | After anesthesia | After exercise |
| 8 normals | 100 | 103 | 107 | 108 | 110 |
| 6 splenectomized | 100 | 105 | 109 | 107 | 110 |
| 6 acute splenectomized | 100 | 105 | 117 | 109 | 118 |
| 6 one-half splenectomized | 100 | 105 | 118 | 109 | 116 |

and unstimulated). These were used for determination of fluid content by dry weight method.

Six splenectomized rabbits, six acute splenectomized rabbits, six half splenectomized rabbits and eight normal rabbits were run in this experiment. Autopsies were performed in each case to determine the extent of operative recovery.

Table 2 gives the results of the hemoglobin changes with anesthesia and exercise in peripheral venous blood and carotid arterial blood. In all cases there was an increased hemoglobin concentration in the arterial blood. After anesthesia the range was 102 per cent to 125 per cent, after exercise 104 per cent to 148 per cent. Venous blood showed an increase in all cases after exercise (range 102 per cent to 126 per cent) but was inconsistent after anesthesia ranging from 93 per cent to 118 per cent. The figures in table 2 are the averages.

Table 3 shows the changes in water content of rabbits' muscles as a result of exercise.

As the result of the peculiarity observed in rabbits' unexercised muscle, namely, that the muscle took up water as well as the exercised muscle, two rabbits were anesthetized and samples from both gastrocnemii were taken. The animals were then allowed to lie anesthetized for forty-five minutes when another sample was taken. The results were the same as in the unexercised muscle in the previous experiments. They had taken up water to the same degree as the other unexercised muscle.

The hemoglobin changes in this experiment parallel those of experiments on non-anesthetized dogs and to about the same degree. There is a noticeable difference in the splenectomized rabbits, namely, that they show a greater degree of concentration than the dogs that were splenectomized. There is also a tendency for the anesthesia to cause a slight concentration of the blood.

The changes in rabbits' muscle are much less than those in the frog. If we average all the groups there is only an increase of 0.3 per cent water

TABLE 3

| RABBITS (AVERAGE OF) | WATER CONTENT OF LATERAL HEAD OF GASTROCNEMIUS IN PER CENT | | | | |
|---|---|---|---|---|---|
| | Normal | | Stimulated difference | | |
| | Before exercise period | After exercise period | After exercise | Normal | Exercised |
| 8 normals | 76.8 | 77.6 | 78.4 | 0.8 | 1.6 |
| 4 splenectomized | 75.9 | 78.5 | 78.6 | 2.6 | 2.7 |
| 6 acute splenectomized | 77.0 | 77.9 | 78.7 | 0.9 | 1.7 |
| 6 one-half splenectomized | 76.2 | 77.5 | 76.9 | 1.3 | 0.7 |

in the stimulated over the resting muscle. That the change in muscle should be small while the change in blood should be large is readily understood if one considers the volumes involved.

METHODS AND PROCEDURES FOR PRODUCING MUSCLE SORENESS AND THE DETERMINATION OF THE CHANGES IN THE VOLUME OF A PORTION OF THE FOREARM AS A RESULT OF THAT EXERCISE. The prerequisites for the study of the production of muscle soreness as described are: muscles not used in the usual pursuits of the individual and muscles easily accessible for measurement. The group decided upon was the pronators of the forearm and hand.

An apparatus suitable for the production of exercise in that particular group of muscles was made (fig. I). It consists of a friction wheel similar to those used on bicycle ergometers. Into one end of the drive shaft of this wheel is fitted the shaft of a ratchet screwdriver. The friction produced in turning the wheel against the brake shoe causes the arm of a

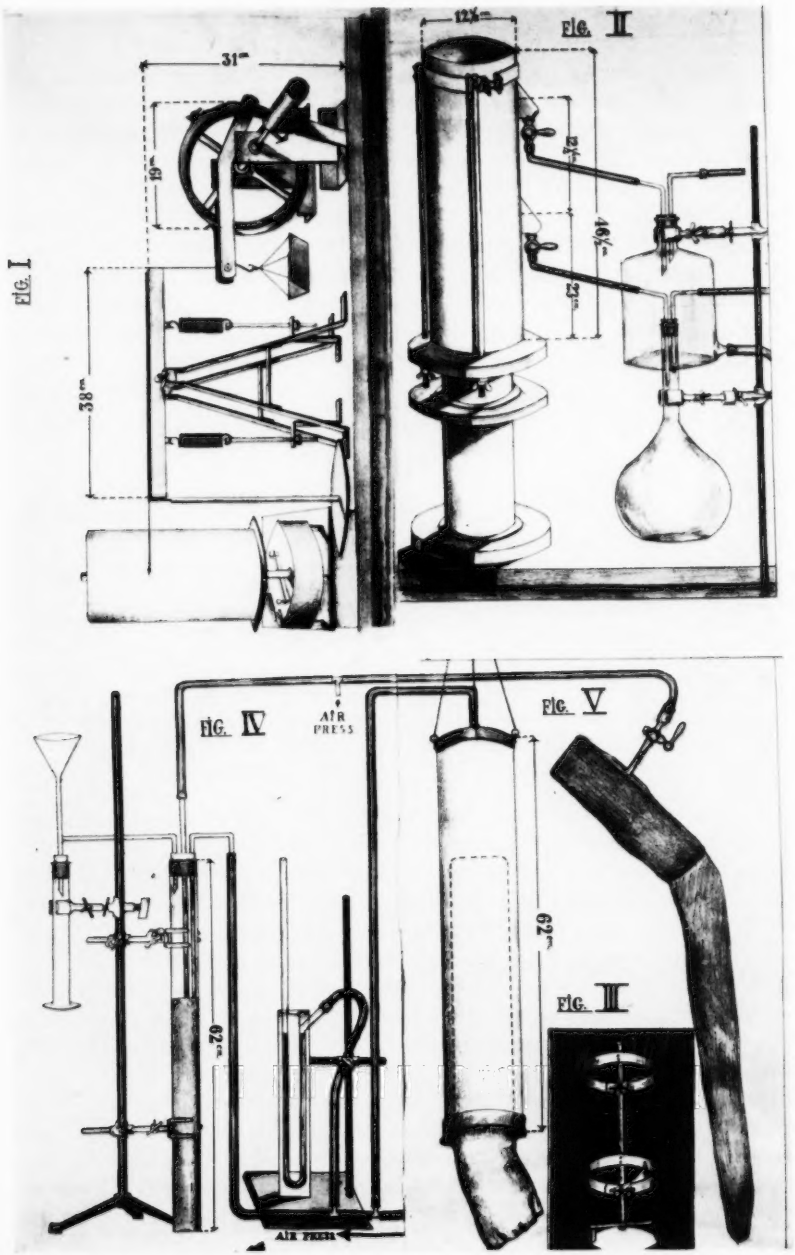
FIG. I
31"
19"
38"
FIG. II
12½"
12"
16½"
12"
FIG. IV
AIR PRESS
79"
AIR PRESS
FIG. V
67"
FIG. III

Figs. 1–5

balance to be drawn down. On the left arm of the balance is a scale pan and extending beyond the arm is a light aluminum pointer which records each stroke on a revolving drum.

The subject was placed in such a position that he might use only muscles desired. The subject was asked to make strokes at the rate of one per second, in time with a metronome. He was also asked to continue beyond his first desire to stop. Once past this period (usually about two minutes in duration) the subject found little difficulty in continuing for periods up to 65 minutes. There were times when strokes were lost due to changes in body position and temporary stops for the adjustment of the hand.

A modified replacement plethysmograph (fig. II) was found to be suitable for measuring changes in the arm. This consists of a brass tube of 12.5 cm. inside diameter and 46.5 cm. deep, closed at one end. Two holes are placed in it, one 23 cm. and the other 35.5 cm. from the bottom, for the purpose of drawing off water at those levels. A cuff was devised for the hand (fig. III). The hand is fitted into the cuff with the fingers under an adjustable bar, after which two copper straps are fastened, one around the wrist and the other one around the fleshy part of the forearm. The copper straps are fixed on an upright rod, thus holding the hand and wrist in a fairly constant position.

The amount of residual water, after the arm was placed in the plethysmograph, was measured by drawing the water off from the top level, then collecting the water to the lower level in a Florence flask by suction. The water was weighed and the volume of the arm calculated by subtracting the water drawn off from the total volume of the cylinder between the holes.

A blood pressure cuff was used to control the amount of venous blood in the arm. The subject was asked to hold his hand over his head for at least 30 seconds so that as much as possible of the venous blood would drain out by gravity. The cuff was then applied and inflated by means of a constant pressure of 220 mm. Hg. maintained by a mercury valve and compressed air. The mercury valve is shown in figure IV.

The series of experiments run by this method are included in the results (table 7), but it was thought that more of the blood and extracellular fluid might be removed from the forearm. This was tried in the following manner: another tube 62 cm. in length and 12.5 cm. inside diameter was made of galvanized iron (fig. V). A lead-in pipe was soldered on

Fig. 1. Ergograph—description in the text.

Fig. 2. Modified plethysmograph—description in the text.

Fig. 3. Cuff for hand—description in the text.

Fig. 4. Mercury valve for maintaining a constant air pressure in the blood pressure cuff and in the compressing tube.

Fig. 5. Compressing tube—description in the text.

the base to accommodate the pressure tubing carrying the compressed air. On the opposite (open) end was attached a piece of light automobile inner tubing about 43 cm. in length. The opposite end of this tubing was sealed, after which it was turned into the rigid tube. The forearm was placed inside the tubing which was to serve as a sleeve. The pressure was varied from 110 to 150 mm. Hg inside the rigid tube (using a mercury valve to hold the constant pressure). The arm was compressed for 30 seconds when the blood pressure cuff, which had previously been put on the arm and forced part way into the rubber sleeve, was inflated to 220 mm. Hg pressure.

TABLE 4

| SUBJECT | DATE | TIME | VOLUME OF ARM | REMARKS |
|---|---|---|---|---|
| | | | cc. | |
| R. W. B. | 3-10 | 9:31 a.m. | 766.9 | Routine work |
| | 3-10 | 9:45 | 766.1 | |
| | 3-10 | 10:12 | 767.5 | |
| | 3-10 | 11:30 | 766.0 | |
| | 3-11 | 7:45 a.m. | 768.6 | Cold |
| | 3-11 | 4:15 p.m. | 759.7 | Marked papers |
| | 3-24 | 7:05 a.m. | 766.4 | Cold |
| | 3-27 | 6:55 a.m. | 757.5 | |
| R. W. C. | 3-16 | 10:00 a.m. | 802.7 | Lab. work |
| | 3-16 | 11:30 a.m. | 801.0 | |
| | 3-16 | 12:00 Noon | 803.3 | |
| | 3-16 | 4:15 p.m. | 804.9 | |
| | 3-16 | 5:35 | 809.7 | Feeling tired |
| | 3-20 | 9:30 a.m. | 813.9 | |
| | 3-23 | 11:05 a.m. | 800.3 | |

The forearm was then removed from the rubber sleeve and the special handcuff placed on it. It was then put into the brass tube for measurement of its volume by the described method.

In all determinations of an individual arm there is not more than 6 cc. range on any given day unless the individual has performed some unusual task during the day. However from one day to the next there may be a maximum change of 20 cc. in the arm volume. It is difficult to account for this change and no evidence is given to explain it. Table 4 shows some of the measured changes without exercise.

*Differences in the volume of the arm under varying conditions.* Before attempting the last experiment several determinations were made on two subjects to observe the change in the volume of the measured portion of the arm under three conditions: 1, with the blood supply normal; 2,

with the arm held over the head for thirty seconds and the blood pressure cuff applied at 220 mm. of mercury pressure, and 3, with the arm in the compressing sleeve for thirty seconds at 122 mm. Hg pressure followed by the application of the blood pressure cuff. The results are recorded in table 5.

*Effect of exercise on the volume of the arm.* 1. *Over six day period.* In this experiment the arm was measured before the exercise (an average of two or more readings none of which varied more than plus or minus 3 grams) after having been held above the head for thirty seconds and the blood pressure cuff applied at 220 mm. Hg pressure. Following this the subject worked on the ergometer and continued until he complained of tiring. The arm was measured immediately after the exercise and afterwards as closely as possible each twenty four hours for the next four or five days.

TABLE 5

*Arm volumes under different conditions*

| SUBJECT | NORMAL ARM VOLUME | ARM WITH BLOOD PRESSURE CUFF AT 220 MM. Hg VOL. OF ARM AFTER VENOUS BLOOD IS DRAINED OFF | DIFFERENCE GRAMS WATER DECREASE IN ARM VOLUME | VOLUME OF ARM AFTER DRAINING OFF VENOUS BLOOD AND COMPRESSION AT 122 MM. Hg | DIFFERENCE GRAMS WATER DECREASE IN ARM VOLUME | REMARKS |
|---|---|---|---|---|---|---|
| | *cc.* | | | | | |
| M. M. W... | 620.9 | 616.1 | 4.8 | 613.7 | 2.4 | Averages from twenty determinations |
| R. R..... | 938.2 | 922.3 | 15.9 | 917.0 | 5.3 | Averages from thirty-five determinations |

Table 6 gives the amounts of work done by the various subjects all of whom experienced some muscle soreness on palpation before the twenty-four hour reading. From this table it can be seen that some of the subjects did from five to ten times the amount of work of others without apparent discomfort during the process.

In every subject studied there has been an increased volume of the arm immediately after exercise. Twenty-four hours after the exercise (table 7), the arms of six subjects were still somewhat larger, five were to all practical purposes normal while one was definitely smaller. By forty-eight hours after the exercise only one of the arms was still large, the others being practically normal or smaller than before exercise.

The subjective signs of soreness are always questionable but when the experimenter palpated the muscles of each forearm in every subject (by a petrissage or deep kneading action) soreness was felt in the exercised

arm by the subjects from 4 to 24 hours after the exercise. An algometer was not immediately available to test the pressure pain sense. W. W. M.

TABLE 6

*Work done on ergometer in producing muscle soreness*

| SUBJECT | NUMBER OF REVOLUTIONS | NUMBER OF STROKES | WEIGHT LIFTED PER STROKE | CALCULATED WORK DONE |
|---|---|---|---|---|
| | | | *grams* | *gram cm.* |
| D. A. C. | 145 | 500 | 800 | $5.8 \times 10^6$ |
| F. W. H. | 214 | 1000 | 750 | $8.0 \times 10^6$ |
| W. W. M. | 46 | 426 | 1900 | $4.4 \times 10^6$ |
| F. Z. | 92 | 542 | 1800 | $8.3 \times 10^6$ |
| M. C. | 71 | 400 | 1235 | $4.7 \times 10^6$ |
| P. H. | 282 | 2443 | 1400 | $2.0 \times 10^7$ |
| L. E. A. | 28 | 290 | 1500 | $2.1 \times 10^6$ |
| H. P. | 103 | 935 | 1600 | $8.2 \times 10^6$ |
| M. W. | 346 | 1565 | 1200 | $2.1 \times 10^7$ |
| R. W. B. | 357 | 1114 | 1200 | $2.1 \times 10^7$ |
| R. W. C. | 155 | 1150 | 1200 | $8.0 \times 10^6$ |
| F. B. | 418 | 1068 | 1200 | $2.5 \times 10^7$ |

TABLE 7

*Changes in volume of forearm following exercise*

| SUBJECT | VOLUME NORMAL | VOLUME AFTER EXERCISE | DIFFERENCES OF WATER FROM NORMAL | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Immediately after | 24 hours after exercise | 48 hours after exercise | 72 hours after exercise | 96 hours after exercise | 120 hours after exercise |
| | | | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* |
| D. A. C. | 702.9 | 713.9 | 11.0 | 10.8 | −0.1 | 5.6 | 9.1 | |
| F. W. H. | 713.6 | 730.2 | 16.6 | −3.4 | 0.2 | 6.0 | 5.8 | |
| W. W. M. | 780.5 | 828.0 | 47.5 | 8.9 | 19.3 | 16.6 | 14.4 | 7.4 |
| F. Z. | 754.6 | 784.8 | 30.2 | 5.0 | 2.9 | 16.7 | 1.7 | 1.9 |
| M. C. | 693.2 | 725.3 | 32.1 | −11.8 | −10.5 | −2.6 | −11.5 | 3.1 |
| P. H. | 726.4 | 740.8 | 14.4 | 0.1 | 6.7 | 1.3 | −5.3 | |
| L. E. A. | 676.3 | 707.0 | 30.7 | −1.6 | −3.9 | −1.1 | −0.9 | |
| H. P. | 761.7 | 785.8 | 24.1 | 0.6 | 7.8 | 13.1 | | |
| M. W. | 612.7 | 622.2 | 9.5 | 14.9 | 5.3 | −1.0 | 8.5 | |
| R. W. B. | 778.3 | 796.4 | 18.1 | 0.0 | 3.5 | −0.2 | 0.1 | |
| R. W. C. | 813.1 | 831.3 | 18.2 | 12.0 | −5.1 | 5.1 | 7.4 | 0.9 |
| F. B. | 669 | 683.6 | 14.6 | 12.7 | −9.8 | −0.8 | −5.8 | 0.0 |
| Averages | | | 22.25 | 4.0 | 1.4 | 4.9 | 2.2 | 2.6 |

(table 7) whose arm remained large over a five day period expressed no soreness after the forty-eight hour reading, while M. C. whose arm was smaller after twenty-four hours showed soreness at that time and for

48 hours after. In no case was there a distinct soreness in less than four hours.

2. *Over a period of twelve hours.* In these cases the subject put his arm into the sleeve of the compressing apparatus described above. Pressure was applied at 122 mm. Hg for thirty seconds following which the blood pressure cuff was applied. The arm was then measured in the plethys-

TABLE 8

*Work done on ergometer in producing muscle soreness*

| SUBJECT | NUMBER OF REVOLUTIONS | NUMBER OF STROKES | WEIGHT LIFTED PER STROKE | TIME OF WORK | STROKES PER MINUTE | CALCULATED WORK DONE |
|---|---|---|---|---|---|---|
| | | | *grams* | *minutes* | | *gram cm.* |
| R. R.[1] | 502 | 2925 | 900 | 60 | 49 | $2.2 \times 10^7$ |
| R. R.[2] | 413 | 1385 | 1280 | 40 | 35 | $2.6 \times 10^7$ |
| M. M. W.[1] | 1021 | 2237 | 800 | 55 | 41 | $4.1 \times 10^7$ |
| M. M. W.[2] | 480 | 1721 | 1280 | 45 | 38 | $3.0 \times 10^7$ |
| R. W. B.[1] | 443 | 2172 | 1270 | 60 | 36 | $2.8 \times 10^7$ |
| R. W. B.[2] | 550 | 2475 | 2100 | 45 | 55 | $5.7 \times 10^7$ |

TABLE 9

*Changes in volume of forearm following exercise*

| SUBJECT | VOLUME NORMAL | VOLUME IMMEDIATELY AFTER EXERCISE | DIFFERENCES OF WATER FROM NORMAL | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Immediately after exercise | One hour after | Two hours after | Three hours after | Six hours after | Nine hours after | Twelve hours after | Twenty-four hours after |
| | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* |
| R. R.[1] | 922.3 | 939.8 | 17.5 | 1.1 | −1.7 | 9.7 | 0.0 | 0.0 | −6.1 | −5.3 |
| R. R.[2] | 916.4 | 926.8 | 10.4 | −2.8 | 1.8 | 10.5 | −16.6 | 1.3 | −4.6 | 10.6 |
| M. M. W.[1] | 611.6 | 620.7 | 9.1 | −0.7 | 2.8 | −2.4 | −3.0 | −13.1 | −3.1 | 2.1 |
| M. M. W.[2] | 613.0 | 634.6 | 21.6 | 8.9 | 7.4 | 8.5 | −0.5 | 3.0 | −2.0 | 4.7 |
| R. W. B.[1] | 766.4 | 790.9 | 24.5 | 10.9 | 10.3 | 10.9 | −0.8 | −1.4 | −0.1 | |
| R. W. B.[2] | 757.5 | 782.9 | 25.4 | 14.4 | 8.3 | 16.3 | 12.5 | 12.0 | 9.8 | |
| Averages | | | 18.1 | 5.3 | 4.8 | 8.9 | −1.4 | 0.3 | −1.0 | 3.0 |

mograph as described previously. The subject was then put to work on the ergometer at the rate of about thirty strokes per minute for one hour. Following the exercise the arm was compressed and measured immediately and thereafter at intervals of one, two, three, six, nine, and twelve hours, and in some cases twenty-four hours.

Table 8 shows a somewhat regulated exercise in which it was endeavored to give a light load at one time and a heavy load at another time. In that experiment M. M. W. was a short thin type of individual, R. W. B.

was of medium build and R. R. was the big heavy type. In both the light and heavy work M. M. W. showed less discomfort than R. R. Subject R. W. B. was able to do more work with a heavy load and at a faster rate than either of the other two subjects. From physical appearance R. R. should have done more work because of his better condition but that did not turn out to be the case.

These subjects, in which compression of the arm took place, all showed larger arms immediately after the exercise (table 9). However, practically each case was different in its recovery to normal size. R. R. had returned to normal within one hour but showed another increase three hours after the exercise. In the experiment in which the work had been done in a shorter period this subject showed an increased size of the arm twenty-four hours later.

M. M. W.[1] showed recovery within one hour in the experiment of less work. M. M. W.[2] with more work required a longer period of recovery (sometime between the third and the sixth hour). R. W. B.[1] with little work showed recovery between the third and sixth hour after exercise but in the shorter heavier work (R. W. B.[2]) recovery was incomplete after twelve hours.

Here again muscle soreness appeared in the subjects from 4 to 12 hours after the exercise was over. These results, like those of the previous group of results, seem again to show no relationship between work, the size of the arm, the amount of change and the amount of muscle soreness. It is difficult in the light of these results to say that this muscle soreness is due to any of the usual explanations for this condition. It is probably best to merely say that it is possible to get muscle soreness which has no relationship to the size of the arm.

Discussion. During exercise, if it is at all severe, fluid leaves the blood and enters the muscle. Except for the results recorded for frog's muscle in this paper we know of no other direct results on the length of time fluid remains in the muscles of unanesthetized animals. Barcroft and Kato (1915) found in anesthetized dogs that, after stimulation, the muscle was not back to normal in eight hours but a good deal of this time must have been due to the anesthetic since the oxygen use did not return to normal for five or six hours.

There are, however, a number of observations on the return of the blood to normal. Schneider and Havens (1914) found the blood normal in from one to two hours after the exercise. Scott, Herrmann and Snell (1917) found that after stimulation of the sciatic nerve the blood returned to normal in from ten to fifteen minutes. Schneider and Crampton (1935) found the blood back to normal in a few minutes after moderate exercise. Keys and Adelson (1936) found the calcium and plasma protein back to normal in from thirty to seventy (average forty-five) minutes after running

on a treadmill for one minute. Hill, Long and Lupton (1924) found the lactic acid of blood practically normal after sixty minutes. We also have some, as yet, unpublished data on athletes showing their blood count had returned to normal in about forty-five minutes. There is thus no evidence from the study of blood of any prolonged edema of the muscle. A little calculation shows that the functional edema of exercise could not be considered a severe one. The greatest concentrations of blood for man we can find in the literature are those of Hawk (1904) who found increases in the red count of 25 and 26 per cent in subjects who ran 100 yards and played water polo for three minutes. A passage of 500 cc. of fluid from the plasma would produce this concentration of the blood but this amount of fluid entering the muscles would hardly increase their water content by 1 per cent since so many of the muscles are involved in those exercises.

The only other theory we know put forward to explain the delayed soreness of muscle is that of Hough (1902) who believes the muscle fibres are injured. However, it seems to us that the pain should show up sooner and be more localized, i.e., not over the entire muscle if the muscle was injured in the ordinary sense. It seems to us that the present theories of delayed muscle soreness (edema or injury) are not supported by facts and that when the explanation of this soreness is found it may be related to alterations in the colloid due to altered electrolyte in the muscle.

## SUMMARY

1. Exercise if severe produces an immediate concentration of blood through loss of water from the blood stream. The spleen plays very little part in the concentration as far as can be determined from these experiments. It is probable that the blood returns to normal in about an hour or less.

2. Frogs' muscle gained water in appreciable amounts after exercise and did not lose that gained water for a number of hours. Rabbits' muscle took up much less water than frogs' muscle.

3. The unexercised muscle of rabbits gained water as the result of the anesthesia.

4. A method of producing muscle soreness and measuring volume changes in the arm is described in detail.

5. Human arms increase in volume as a result of exercise. Part of this increase is due to the active hyperemia that accompanies exercise but in the experiments in which the arms were compressed there was still some increase in the volume of the arm.

6. Human arms which show an increase in their volume return to normal volume in from six to twenty-four hours.

7. Muscle soreness may, however, come on after the arm has returned to normal volume and also may persist after the arm has returned

to normal volume. It would seem, therefore, that although there are vascular and lymph changes in muscle as a result of exercise, the type of muscle soreness described in this paper is not due to imbibition of water and lasts too long to be due to metabolic products accumulated in the muscle.

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# WEIGHTS OF ADRENAL GLANDS IN RATS FED DIFFERENT AMOUNTS OF SODIUM AND POTASSIUM

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Received for publication February 4, 1938

When the rat is subjected to stress such as work, disease, trauma, the injection of certain compounds and toxins, the extremes of temperature, and so forth, the cortices of the adrenal glands increase in size, but when a large excess of cortin is administered to the rat, the cortices of the adrenal glands decrease in size (6). The size of the adrenal glands in the rat is determined in part, at least, by the physiologic requirements for its hormone.

It has been shown in many laboratories (1, 2, 3, 4, 5, 7) that the adrenalectomized animal is responsive to the intake of sodium and potassium in the diet. Potassium is highly toxic to the adrenalectomized animal. A diet which is low in potassium and high in sodium chloride may maintain life indefinitely when the animal is kept free from stress. Zwemer and Truszkowski (8) have presented evidence that the well known variations in mortality among adrenalectomized rats studied in various laboratories may be due to differences in the sodium and potassium content of the diet. We have observed that adrenalectomized rats may show an incidence of survival of more than 90 per cent when they are fed diets high in sodium and low in potassium, and that an identical diet high in potassium and low in sodium produced a mortality rate of more than 90 per cent when the other experimental conditions were constant.

We have determined the weights of the adrenal glands of unilaterally adrenalectomized rats which had been fed different amounts of sodium and potassium and have compared them to weights of adrenal glands which had been caused to hypertrophy by stress.

METHODS. All of the animals used in these experiments were male rats of the Wistar strain which weighed from 180 to 190 grams at the time of the operation. The left adrenal gland was removed from each animal. The basic diet was made up of the following constituents: commercial casein (25 per cent), lard (18 per cent), unsalted butter (7 per cent), sucrose (25 per cent), cracker meal (22 per cent), dried brewers' yeast (2 per cent), and bone ash (1 per cent). The diet contained 0.2 per cent sodium and

0.06 per cent potassium. Sodium chloride and potassium dihydrogen phosphate were the salts added to the diet as described.

EXPERIMENTS AND RESULTS. In experiment one, the left adrenal gland was removed from forty-eight rats. They were closely matched

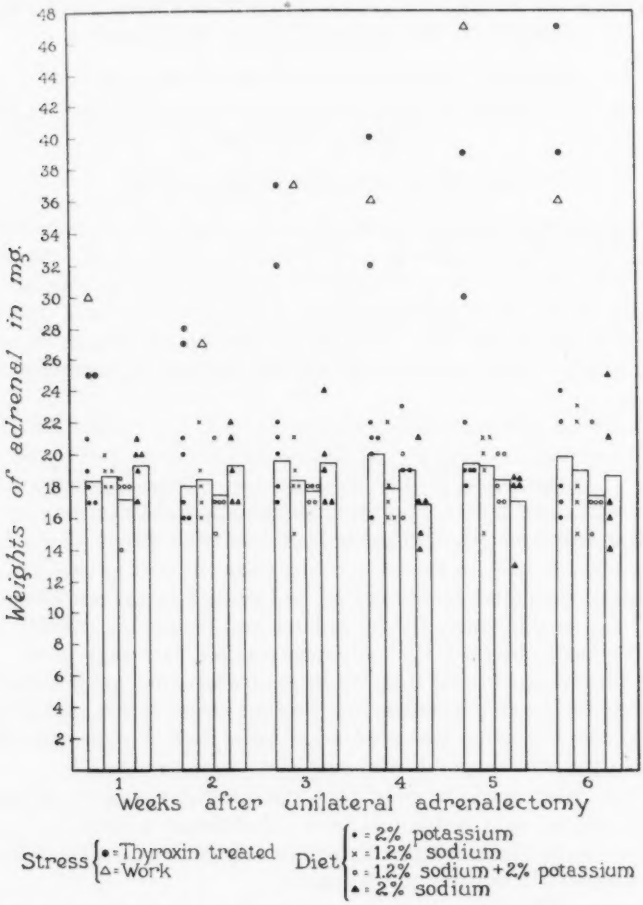


Fig. 1. Weights of adrenal glands of rats fed different amounts of sodium and potassium and weights of adrenal glands of rats subjected to stress.

into four groups. Group A received the basic diet but no additional sodium or potassium, group B received an added 1 per cent of potassium, group C received an added 0.6 per cent of sodium, and group D received an added 1 per cent of potassium and 0.6 per cent of sodium. Two animals

from each group were killed at weekly intervals for six weeks. At necropsy the adrenal glands were weighed to the nearest milligram and all values were found to be within the range of normal variability for the glands of unilaterally adrenalectomized rats.

In experiment two, the left adrenal gland was removed from 120 rats. They were closely matched into four groups. Group E received the basic diet which contained an additional 2 per cent of potassium; group F received an additional 1.2 per cent of sodium, group G received an additional 1.2 per cent of sodium and 2 per cent of potassium and group H received an additional 2 per cent of sodium.

Five animals from each group were killed at weekly intervals up to six weeks. The data on the weights of the adrenal glands which were removed at necropsy are summarized in figure 1. All of the animals showed a gain in body weight following operation. Some of the animals that received diet E showed smaller gains in body weight than animals of the other groups.

In experiment three, the left adrenal gland was removed from twelve rats which were treated with 0.2 mgm. of thyroxine given daily by subcutaneous injection. Two animals were killed at weekly intervals for six weeks. The weights of the adrenal glands are summarized in figure 1. All of these animals showed either a loss of body weight or very small gains over the initial weight.

In experiment four, the left adrenal gland was removed from six rats. They were then required to run for twelve hours each day in a motor driven cage. The distance covered each day was approximately four miles. One rat was killed at weekly intervals for six weeks. The data on adrenal weights are summarized in figure 1. All of these rats showed a loss in body weight during the experimental period.

COMMENT. For adrenalectomized animals it has been clearly established that the maintenance requirements for cortin are reduced when the dietary intake of potassium is low and the intake of sodium is high. When the intake of potassium is high and the intake of sodium is low the maintenance requirements for cortin are increased. This correlation between the intake of electrolytes and the maintenance requirement for cortin has also been found to apply in the treatment of Addison's disease (5).

In the experiments in which one adrenal gland was left intact it is apparent that the physiologic requirements for cortin were not sufficiently reduced or increased by wide variations in the dietary intake of electrolytes to produce significant changes in the weights of the adrenal glands. The stress produced by work and by the administration of thyroxine brought about a marked hyperplasia of the adrenal gland.

These results have been anticipated by the unpublished results of an investigation by H. B. Friedgood and Maes of the Department of Physi-

ology, Harvard Medical School, in a study of the size of the adrenal glands of guinea pigs. Female animals were matched on a weight basis. One group received a basic diet of vegetables, the second group was fed vegetables which had been dipped in a concentrated solution of sodium chloride and the third group was fed vegetables after they had been dipped in a concentrated solution of potassium chloride. In addition, sodium chloride and potassium chloride were injected into the respective groups. After a period of eighteen days necropsy was performed. No significant differences were found between the weights of the adrenal glands in the control and experimental groups.

SUMMARY

When male rats were unilaterally adrenalectomized and given diets which contained varying amounts of sodium and potassium, the average weights of the remaining adrenal glands were not significantly different among the experimental groups. Similar animals were treated with thyroxine and others were forced to work in revolving cages. The weights of the adrenal glands of the animals subjected to these forms of stress were greatly increased.

We wish to acknowledge the courtesy of Doctor Friedgood and Doctor Maes for the opportunity to discuss their experimental results.

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# EFFECT OF AVITAMINOSIS A ON THE BLOOD PICTURE OF ALBINO RATS

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Received for publication January 17, 1938

Several investigators have studied the changes occurring in the blood of rats on a vitamin A deficient diet. Among these are Sure, Kik and Walker (2), Turner and Loew (3), Crimm and Short (1). All have been especially concerned with changes in the blood picture of young rats with acute avitaminosis A.

There are, however, phases of this problem which have received little attention: one, the effect on the blood picture of successive depletions of vitamin A followed by recovery; another, the effect of a diet low in vitamin A over a long period of time.

*Plan of study.* The plan of this investigation was to study the blood picture of *a*, young rats during two vitamin A depletion-recovery periods, and *b*, adult rats fed for a long time on a diet low in vitamin A.

METHODS. 1. *Depletion of young rats.* The standard technique was used in preparing rats for vitamin A studies. Rats of uniform weight were placed on a vitamin A deficient diet[1] when weaned at 21 days. The control animals received daily in addition to the basal diet one drop of carotene in cottonseed oil (500 U. S. P. units of vitamin A). Distilled water and food were given *ad libitum*. After gross symptoms of avitaminosis A developed in the experimental animals, each rat was given 5 or 6 drops of carotene in cottonseed oil (2500 to 3000 units of vitamin A). This was administered over a period of 4 days. When recovery was apparent the rats were again subjected to a depletion period and the above procedure repeated.

2. *Depletion of adult rats.* Female rats six months or more of age previously fed diets containing adequate amounts of vitamin A were placed on a diet low in vitamin A. Many of these had borne several litters, but all were in a good state of nutrition.

| | *per cent* |
|---|---|
| [1] Purified casein | 18 |
| Salt mixture—Osborne and Mendel (1919) | 4 |
| Irradiated yeast | 10 |
| Corn starch | 67 |
| Sodium chloride | 1 |

*Examination of the blood.* Blood was secured by tail cutting and no rat was used a second time until his tail had healed thoroughly. Total red and white cell counts, hemoglobin estimations, and differential counts were made when the rats were placed on the experimental diets. Each week thereafter blood studies were made on selected rats from the group.

The cell counts were made with a Levy counting chamber and the percentage of hemoglobin determined with a Dare hemoglobinometer. The blood was considered normal when it contained 7 to 9 million erythro-

TABLE 1

*Summary of blood picture according to age of normal albino rats (Average of 46 males and 48 females)*

| AGE | HEMOGLOBIN | TOTAL WHITE BLOOD CELLS | TOTAL ERYTHROCYTE | PER CENT | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Polymorphonuclears | Stab forms | Large lymphocytes | Small lymphocytes | Large monocytes | Myelocytes | Unclassified |
| *weeks* | *grams per 100 cc.* | | *millions* | | | | | | | |
| 3 | 12.3 | 5,521 | 6,5 | 20 | 2 | 2 | 72 | 2 | 1 | 1 |
| 5 | 12.8 | 5,200 | 6,7 | 22 | 2 | 2 | 70 | 2 | 2 | 0 |
| 6 | 13.1 | 5,000 | 7,0 | 25 | 1 | 3 | 69 | 1 | 1 | 0 |
| 7 | 13.1 | 5,400 | 7,0 | 23 | 1 | 3 | 69 | 1 | 1 | 0 |
| 8 | 12.7 | 5,200 | 6,8 | 20 | 1 | 1 | 76 | 1 | 1 | 0 |
| 9 | 13.7 | 5,500 | 7,0 | 18 | 3 | 2 | 75 | 1 | 1 | 0 |
| 10 | 13.5 | 6,700 | 7,5 | 16 | 4 | 1 | 65 | 1 | 3 | 0 |
| 12 | 11.7 | 6,000 | 7,9 | 27 | 2 | 2 | 64 | 2 | 3 | 0 |
| 15 | 13.7 | 5,800 | 8,0 | 23 | 1 | 1 | 73 | 1 | 0 | 1 |
| 18 | 12.8 | 8,000 | 8,5 | 22 | 1 | 3 | 73 | 1 | 0 | 0 |
| 20 | 13.8 | 7,700 | 8,4 | 20 | 2 | 4 | 70 | 1 | 1 | 2 |
| 23 | 13.7 | 7,375 | 9,3 | 22 | 2 | 2 | 78 | 2 | 2 | 0 |
| 26 | 14.5 | 7,800 | 9.2 | 28 | 2 | 3 | 65 | 1 | 1 | 0 |
| 30 | 14.5 | 7,000 | 8,9 | 25 | 0 | 1 | 73 | 2 | 0 | 0 |
| 38 | 14.7 | 7,700 | 9,2 | 33 | 1 | 4 | 60 | 0 | 1 | 1 |
| 40 | 15.9 | 8,800 | 8,7 | 29 | 3 | 4 | 63 | 1 | 0 | 0 |
| 43 | 15.9 | 8,100 | 8,8 | 33 | 4 | 4 | 54 | 2 | 1 | 1 |
| 46 | 15.5 | 8,000 | 7,9 | 34 | 2 | 3 | 55 | 1 | 3 | 2 |

cytes per cmm. and 5000 to 8000 leucocytes per cmm. One hundred white cells on each of three smears stained with Wright's stain were used in making the differential counts. Observations were made to determine abnormalities in cell shape or any unusual or degenerate bodies in the blood. Young granulocytes were distinguished from the young lymphocytes by the peroxide reaction. The technique of Goodpasture was followed.

RESULTS AND DISCUSSION. *Vitamin A deficient rats.* The results presented in table 1 and figures 1 and 2 cover the blood pictures of 300

albino rats which included approximately 200 rats fed for different periods of time on diets low or deficient in vitamin A, together with 94 litter mate controls.

*A. The blood pictures of young rats during two vitamin A depletion-recovery periods.* When the rats were 6 weeks old and had been on the vitamin A deficient diets for approximately three weeks changes occurred in the blood concurrent with the first symptoms of xerophthalmia. These changes consisted of an increase from 7400 to 9200 in the leucocytes, an increase of from 22 to 42 per cent in neutrophile polymorphonuclears, and a drop in lymphocytes from 70 to 50 per cent (fig. 1). At the end of the fourth week the polymorphonuclears had increased to 70 per cent

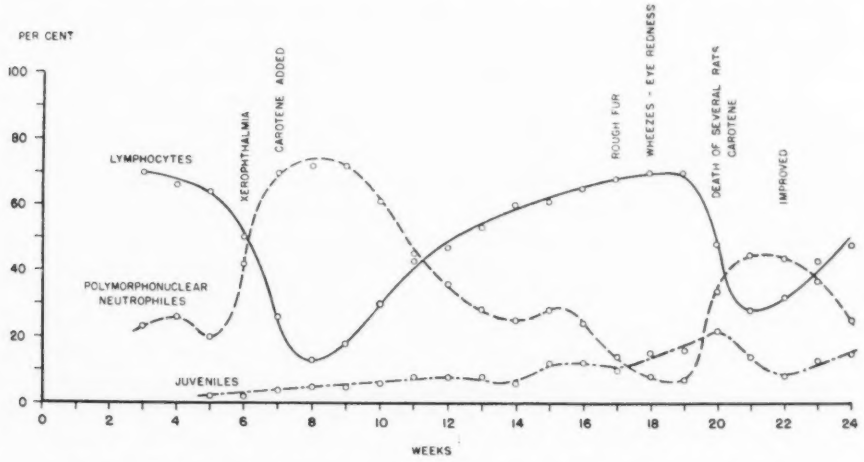


Fig. 1. Variation in percentage of lymphocytes and polymorphonuclear neutrophiles in avitaminosis A.

and the lymphocytes decreased to 16 per cent; the average white cell count had now reached 16,000, an increase of 7000 per cmm. over the controls. Seven days later 74 per cent of the white cells were polymorphonuclears and only 12 per cent were lymphocytes. With the addition of 2500 to 3000 U. S. P. units of vitamin A administered over a period of four days there was a gradual improvement in the general condition, and in about 6 weeks there was a return to normal in respect to the ratio of polymorphonuclears to lymphocytes.

Following recovery the rats were again depleted. But during the first weeks of this depletion a significant change occurred in respect to the number of large lymphocytes. At the beginning of the experiment the large lymphocytes constituted an average of 6 per cent of the total

leucocyte count (fig. 2) but during the second depletion the large lymphocytes increased to 16 per cent although the animals showed no external symptoms or signs of deficiency. All animals still appeared to be in a fair state of nutrition. Gains in weight had been made; the eyes were free from infection and the hair had become smooth and glossy. No gross symptoms of avitaminosis were noted for several weeks. But at the beginning of the 14th experimental week they again showed external symptoms of malnutrition as indicated by lack of vigor, flabby musculature, and rough fur. Several days later respiratory disorders, such as sniffles, wheezes and a discharge from the nose were present to a greater or less degree in all animals. A slight redness about the eyes was the only symptom of xerophthalmia.

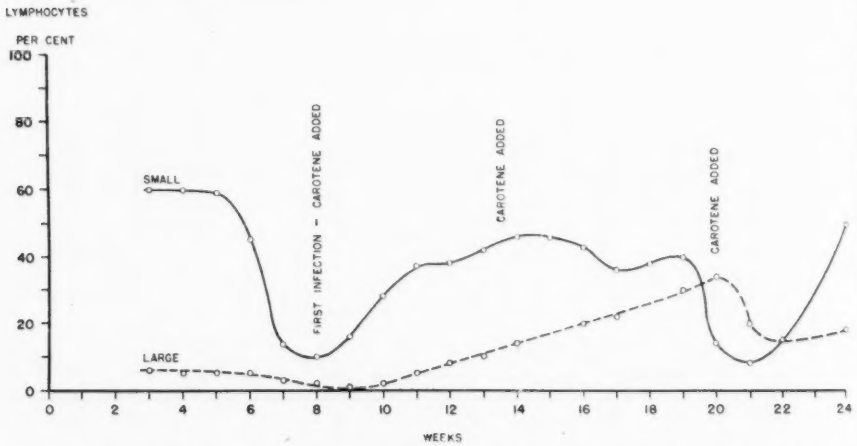


Fig. 2. Variation in percentage of large and small lymphocytes in avitaminosis A.

A comparison of the differential counts during the infection concurrent with the first and second vitamin A deficient periods showed two very different pictures. In the first case the blood showed leucocytosis, there being an average leucocyte count of 16,500; but in the second infection there was a leucopenia, as only 3000 leucocytes were present. In the second infection the percentage of polymorphonuclears continued to drop until during the 18th week it reached 8 per cent of the total count. However, the juvenile forms, stabs, myelocytes, and myeloblasts increased and constituted 21 per cent of the total count (fig. 1). The unusual number of these young forms indicated damage to the bone marrow. This was verified by examination of the marrow in the long bones. The usual finding was a gelatinous degeneration of the marrow but occasionally an almost

complete replacement by fibrous stroma had occurred. Throughout this depletion period the lymphocytes remained relatively high; the large lymphocytes continued to increase until they constituted 32 per cent of of the total count.

Many leucocytes showed degeneration; the lymphocytes became irregular both as to size and shape and bizarre forms were not uncommon. The myelocytes were often vacuolated and distorted. The greatest amount of degeneration however, was observed in the polymorphonuclears. Many of them showed toxic granules and vacuolated cytoplasm, while in others the cytoplasm appeared as faintly stained strands, the nucleus was fragmented and granular and the cell wall indistinct and broken. As the deficiency became more acute, it was not uncommon to find a naked nucleus, the cell wall and cytoplasm entirely missing. Changes were also noted in the erythrocytes. Many of these cells took a basophilic stain and younger forms such as normoblasts and other immature red cells were seen occasionally. Crescent bodies were now present.

More than half of the animals died of respiratory diseases during the 18th and 19th weeks. Carotene was then given. Seven days following the addition of carotene no changes were found in the blood picture, but the general appearance and vigor of the rats improved. During the following week the polymorphonuclears increased to 35 per cent and ten days later they reached a maximum of 45 per cent. The young forms also increased at this time a maximum of 20 per cent being reached. While there was a slight drop in the percentage of large lymphocytes the ratio of large to small lymphocytes remained high. During the following two weeks recovery occurred with improvement in the blood picture.

*Adult rats on a diet low in vitamin A.* After the adult rats had been transferred from stock food to diets very low in vitamin A it required approximately three months for symptoms of malnutrition to appear. The blood picture, taken on successive weeks, showed no changes in the differential count for the first two months. During the third month the animals began losing weight and the fur was rough. During the fourth month a large number of the animals developed respiratory diseases. The differential count at this time showed an increase in both polymorphonuclears and in large lymphocytes. The infection was manifested by inflammation of the eyes, wheezes and sluggishness. As the avitaminosis progressed the polymorphonuclear count dropped instead of increasing as one would expect and the young forms increased. Juvenile cells, myelocytes and even a few myeloblasts were found. There was now a marked rise in large lymphocytes and a few lymphoblasts were seen. The large lymphocytes in some cases made up 60 per cent of the total leucocyte count.

After about 5½ months the animals began to succumb to respiratory infections. At this time the average differential count of 100 one-year-old females was as follows:

| | *per cent* |
|---|---|
| Polymorphonuclear | 10 |
| Juveniles | 6 |
| Stabs | 8 |
| Small lymphocytes | 6 |
| Large lymphocytes | 60 |
| Unclassified | 10 |

Many of the cells were fragmented and degenerate at this time, and classification became difficult. The unclassified cells often constituted more than ten per cent of the total count.

The outstanding changes in the blood picture were: 1, marked decrease in polymorphonuclears; 2, an increase in the number of large lymphocytes; 3, an increase in the number of young cells, and 4, degeneration, especially of the granulocytes.

Carotene was given and in three weeks the blood picture showed a return to normal. Again the outstanding change was observed in the lymphocytes. The large lymphocytes comprised about 50 per cent of the total leucocyte count at the peak of the infection but when carotene was added to the diet they dropped in three weeks to 20 per cent of the total count. It should be added, however, that even on a diet high in vitamin A an abnormally high percentage of large lymphocytes persisted following the disappearance of any physical signs of vitamin A deficiency.

SUMMARY. The following constant and characteristic changes have been found in the blood picture of 1, young rats during two vitamin A depletion-recovery periods, and 2, adult rats fed a long time on a diet low in vitamin A.

*Young rats, first infection.* 1, increase in total leucocytes (leucocytosis); 2, increase in polymorphonuclears (from 22 to 74 per cent); 3, on addition of carotene there was a remission of physical signs of avitaminosis and a return to normal of the blood picture.

*Young rats, second infection.* 1, decrease in leucocytes (leucopenia); 2, decrease in polymorphonuclears (8 per cent of total count); 3, increase in stab forms and myelocytes (8 per cent to 22 per cent); 4, increase in large lymphocytes from 6 to 30 per cent; 5, degeneration and vacuolation of myelocytes and polymorphonuclears.

*Adult rats.* 1, marked decrease in polymorphonuclears (22 to 10 per cent); 2, an increase in large lymphocytes from 6 to 60 per cent; 3, an increase in young forms both lymphocytes and granulocytes; 4, degeneration, especially of the granulocytes.

## CONCLUSION

A blood picture with a low polymorphonuclear count, an increase in juvenile cells, and an increase of large lymphocytes over the small, is a constant finding in vitamin A deficiency in rats and should be considered in any study when such a blood picture is found.

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# THROMBIN, A PROTEOLYTIC FIBRINOGENASE

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Received for publication December 6, 1937

Although clotting of fibrinogen by thrombin has been regarded by Hammarsten (1), Mills (2), Mellanby (3), and others as an hydrolysis, this theory is by no means universally accepted. The work here presented gives additional evidence of the cleavage of fibrinogen by thrombin.

EXPERIMENTAL. I. *The nitrogen in fibrin formed is less than in the fibrinogen transformed.* Fibrinogen was prepared from fresh, citrated horse plasma by repeated precipitation with equal volumes of saturated sodium chloride and re-solution in distilled water. Equal amounts of this material in rather dilute solution were placed in each of two tubes. One tube was then heated to 58°C. for thirty minutes and the coagulum filtered and washed repeatedly with 8 per cent sodium chloride. To the other tube a very small amount of thrombin, prepared by the method of Mellanby (3), was added and the contents completely defibrinated. The serum from this clot when added to additional fibrinogen clotted it in each case, thus proving the sufficiency of the amount of thrombin added. The fibrin removed was washed as had been the coagulum obtained by heat and the two were analyzed for nitrogen by the gasometric micro-Kjeldahl method of Van Slyke (4).

The results obtained by this procedure in a series of determinations are expressed in table 1 and show that the fibrin obtained from fibrinogen contained less nitrogen than the heat-coagulated fibrinogen. It indicates that fibrinogen lost about 9.6 per cent of its nitrogen upon conversion to fibrin. These results are very similar to those of Hsü and Wu (5) and indicate the hydrolysis of fibrinogen into fragments very dissimilar in size, rather than into two almost equal parts as Schmiedeberg (6) believed.

II. *There is an increase in number of free amino groups when fibrinogen clots.* Further confirmation of the hydrolysis of fibrinogen was sought by the determination of the number of free amino groups before and after clotting. Sörenson's formaldehyde titration method was used for this determination as the method of Van Slyke (7) proved unsatisfactory.

Samples of citrated fibrinogen were diluted to the point at which they would form only a weak clot which could be broken up with a glass rod. Equal parts of such solutions were pipetted into separate breakers and to

one of these sufficient calcium chloride was added to cause clotting. With the beakers kept covered as much as possible Sörenson titrations were run on both samples using 0.005 N acid and base. The clotted material was broken up as completely as practicable and the final, rather than the initial, end point was read. Table 2 gives the results of a series of these determinations and shows that an increase of about 4 to 5 per cent in free amino groups takes place when fibrinogen clots. Each titration value represents the average of two titrations on identical samples, the readings of which varied by not more than 0.25 cc.

TABLE 1

*Loss of nitrogen of fibrinogen upon conversion to fibrin*

| SAMPLE | FIBRINOGEN NITROGEN | FIBRIN NITROGEN | NITROGEN LOST |
|---|---|---|---|
| | *mgm.* | *mgm.* | *per cent* |
| 1 | 1.92 | 1.71 | 10.94 |
| 2 | 2.81 | 2.59 | 7.83 |
| 3 | 2.65 | 2.40 | 9.43 |
| 4 | 2.17 | 1.91 | 11.98 |
| 5 | 2.46 | 2.27 | 7.72 |
| Average..... | | | 9.58 |

TABLE 2

*Sörenson titration values of fibrinogen before and after clotting*

| SAMPLE | TITRATION BEFORE CLOTTING | TITRATION AFTER CLOTTING | INCREASE |
|---|---|---|---|
| | (*cc. NaOH*) | (*cc. NaOH*) | *per cent* |
| 1 | 24.32 | 25.94 | 6.25 |
| 2 | 21.00 | 22.39 | 6.21 |
| 3 | 34.17 | 35.43 | 3.56 |
| 4 | 23.64 | 24.58 | 3.83 |
| 5 | 32.79 | 34.20 | 4.12 |
| Average..... | | | 4.79 |

III. *The further hydrolysis of fibrinogen by thrombin.* The re-solution of fibrin in sterile dilute salt solutions was observed and described by Dastre (8), who called attention to the similarity of the spontaneous lysis of fibrin to its digestion by trypsin. Haugardy (9) has shown fibrinolysis to be a hydrolytic process and believes thrombin to be the enzyme which causes it.

Fibrinogen as well as fibrin undergoes changes upon standing in sterile solution as is shown by its loss of ability to clot on the addition of thrombin and the appearance of a variable amount of insoluble precipitate. That

such changes might be due to the presence of amounts of thrombin too small to cause clotting is entirely possible. In such a case the lytic products of both fibrin and fibrinogen might be expected to be very similar in nature whereas if the denaturation of fibrinogen were due to a different factor the products obtained would probably be different. These products were compared by the methods described below.

Fibrinogen was prepared as previously described. A part of this was further purified by the method of Mills and Mathews (10) with freshly precipitated tri-calcium phosphate, and then with animal charcoal by the method of Sumner (11). This preparation is here designated as purified fibrinogen. It did not clot within twelve hours when incubated at 37°C. with calcium chloride and cephalin, but clotted in less than five minutes under the same conditions when a small amount of prothrombin, prepared by the method of Mellanby (12), was added. This showed that it had been freed entirely or almost entirely from prothrombin (serozyme). By its clotting activity the removed prothrombin was readily detected in the tri-calcium phosphate precipitate and to a lesser extent in the charcoal.

Fibrin was prepared by allowing a small quantity of Mellanby's thrombin to clot the ordinary preparation of fibrinogen. About one part of thrombin to eight hundred of fibrinogen, on the basis of dry weights was used. The three preparations—fibrin, fibrinogen, and purified fibrinogen —were covered with toluene and incubated at 37°C. At intervals samples were removed for the determination of fibrinogen by salt precipitation, by heat coagulation, and by clotting activity. The amount of fibrinogen still precipitable by salt was determined by adding to a portion of each solution an equal volume of saturated sodium chloride. Opalescence without the formation of a precipitate was not recorded as positive. Clotting power was considered absent if a portion of the solution failed to clot within two hours when incubated with fresh thrombin.

Results. The disappearance of thrombin coagulability of the ordinary preparation of fibrinogen was found to be complete in three days, whereas the purified preparation remained active until the seventh day. Precipitability with half-saturated sodium chloride and coagulability at 56°C., properties of all fibrinogen preparations, remained until the fifth day in the impure fibrinogen, and until the ninth day in the purified material. This greater stability of the more highly purified protein indicates that there had been removed from it by the absorption process a denaturing or proteolytic enzyme of some kind, presumably the prothrombin, which was shown to be removed.

Within thirty minutes of the time of formation of the fibrin in the sample to which thrombin had been added the serum was found to contain substances coagulating at 62°, 66–67°, 72°, 75°, and 84°C. The coagula

at 66 to 67° and 75° were quite heavy while the others were faint but definite. Substances of the same coagulation temperatures were found in the digest of the impure fibrinogen, but they did not appear definitely for over a day and gradually became more pronounced as the material (fibrinogen) coagulable at 56°C. disappeared. In the purified fibrinogen, on the other hand, the appearance of these substances required two days and the coagulum at 72°C. was never definitely present, while that at 84°C. was extremely faint. The substance coagulating at 66 to 67°C. was first discovered by Hammarsten (13) (fibringlobulin) and that coagulating at 75°C. was found by Mills (2).

Whether prothrombin itself may have weak fibrinolytic properties, or whether its activity is dependent upon the formation of thrombin in small amounts by cephalin and a trace of calcium is not known; but in either case the minute quantity of active substance present in the purified fibrinogen indicates that the active substance is an enzyme.

As Hirose (14) had found rather large amounts of an albumose in autolyzed fibrin solutions it appeared probable that the hydrolysis might go even further. The finding of Fuchs and Zahrzewski (15) that non-protein nitrogen increased during clotting strengthened this conclusion. It was therefore decided to analyze both fibrinogen and redissolved fibrin to determine the extent and character of the hydrolysis.

The fibrin and fibrinogen were prepared as previously described and samples allowed to stand under toluol at different temperatures. None of these samples contained, before storage, detectable amounts of nitrogen not precipitable with 2.0 per cent tri-chloroacetic acid. After various periods of time the filtrates from any undissolved materials were analyzed for different hydrolytic products by the method of Wasteneys and Borsook (16). Protein and metaprotein are precipitated by 2 per cent tri-chloroacetic acid; and the metaprotein alone by precipitation at a pH of 6.0. The fraction called protein will include any unchanged fibrinogen. Determinations of nitrogen in the fractions were made by the Van Slyke micro-Kjeldahl procedure. In the case of fibrin autolyzed 205 days at 5°C. and 90 days at room temperature practically complete solution had occurred; and in that autolyzed 7 days at 37°C. much solution had occurred.

Several such series of fractional analyses were made and the results found to be consistent considering the durations and the temperatures of the various incubations. In table 3 are given the results of three series of analyses. Each number is the average of two analytical values differing by not more than three per cent.

These results show very definitely that the breakdown of both fibrin and fibrinogen continues until very simple substances are formed. But the main change, the first splitting, (to form fibrin) is brought about very

promptly by thrombin. That thrombin, or possibly prothrombin, is the substance responsible for the further hydrolysis is strongly indicated by the fact that the velocity of the hydrolysis is dependent upon the amount present of some substance which is precipitated and adsorbed under the same conditions as is prothrombin.

It appears evident that the action of thrombin is one of hydrolysis, and that the formation of fibrin is only an intermediate step in the process. Formation of *appreciable* amounts of fibrin is usually the most rapid step in the hydrolysis; but may not occur, even though the digestion proceeds much further, if thrombin be present in very small amounts.

Whenever fibrin or fibrinogen is allowed to autolyze a more or less heavy flocculent precipitate is formed. In ordinary preparations of fibrinogen the precipitation appears to be complete after one day's incubation at 37°C., but in the purified fibrinogen the precipitate can be detected

TABLE 3

*Nitrogen distribution in autolysates of fibrinogen and fibrin expressed as percentages*

| | AUTOLYZED 205 DAYS AT 5°C. | | | AUTOLYZED 90 DAYS AT ROOM TEMPERATURE | | AUTOLYZED 7 DAYS AT 37°C. | | |
|---|---|---|---|---|---|---|---|---|
| | Fibrinogen | Fibrinogen | Fibrin | Fibrinogen | Fibrin | Fibrinogen | Purified fibrinogen | Fibrin |
| Protein | 30.9 | 32.9 | 22.6 | 42.5 | 23.8 | 67.1 | 76.9 | 36.2 |
| Metaprotein | 32.0 | 30.4 | 11.7 | 10.9 | 11.8 | 17.3 | 19.4 | 23.1 |
| Proteose | 7.0 | 5.6 | 29.7 | 9.5 | 27.9 | 3.9 | 3.1 | 19.8 |
| Peptone | 9.1 | 9.1 | 10.2 | 12.4 | 11.2 | 4.0 | 0.6 | 7.8 |
| Subpeptone | 8.6 | 9.1 | 4.7 | 7.1 | 4.7 | 3.5 | 0.0 | 5.4 |
| Amino and simple peptide | 12.4 | 12.9 | 21.1 | 17.6 | 20.6 | 4.2 | 0.0 | 7.7 |

only after three to ten days' incubation and remains very faint. This observation suggested that the precipitate might be an altered form of thrombin or prothrombin rather than a lytic product of fibrinogen. Examinations of the dried precipitates from solutions of fibrin and ordinary fibrinogen were made but not enough material could be collected from the purified fibrinogen for analysis. All the precipitates examined appeared to be identical. Between 40 and 50 per cent of the precipitate was ether-soluble, whereas only 25 to 35 per cent was soluble in cold alcohol. The extracted precipitate was fairly soluble in dilute sodium hydroxide and contained about 13 per cent of nitrogen. It thus appears that the precipitate consists of a lipide and a protein. Neither the whole precipitate nor the fractions obtained by extracting it with ether showed the clotting activities of prothrombin, thrombin, or cephalin. However, it seems probable that one or more of these substances, in an altered form, was present in the precipitate.

SUMMARY

1. Fibrinogen contains about 9 to 10 per cent more nitrogen than does the fibrin formed from this fibrinogen by thrombin.

2. A small increase in the number of free amino groups takes place during clotting.

3. Fibrin, fibrinogen, and fibrinogen from which practically all the prothrombin had been removed by adsorption, all autolyzed, but at different rates, the fibrin undergoing autolysis most rapidly and the purified fibrinogen least rapidly. All three substances yielded the same products of autolysis as determined by heat coagulation and fractional protein analysis with the exception of the prothrombin-free fibrinogen which was not found to yield fragments smaller than peptones. The substances containing larger amounts of prothrombin not only were digested faster but much more completely.

4. The results strongly indicate that thrombin acts as a proteolytic enzyme in the clotting of fibrinogen; and that the further but slower hydrolysis of fibrin is produced either by thrombin or prothrombin.

*Acknowledgment.* I wish to thank Prof. S. Tashiro and the William S. Merrell Company who placed at my disposal funds for this work. I also wish to thank Professor Mathews and Professor Tashiro for their helpful criticisms and advice.

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# OBSERVATIONS ON THE UTERINE FLUID OF THE RAT

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Received for publication February 3, 1938

It is well known that the secretory activity of the uterine glands of mammals varies considerably at different times in the same mammal. The accumulation of large amounts of uterine fluid seems to be confined to those species of mammals in which the ovaries are surrounded by complete capsules. Long and Evans (1922) first reported the distended condition of the uterine horns of the rat as a result of the collection of uterine fluid during prooestrus and oestrus. Hartman (1923) attributed the distention of the lateral vaginal canals of the opossum to the collection of a clear fluid. Hammond (1927) has shown that the mucous glands of the cervix of the cow secrete the greatest amount before the time of ovulation. Parkes (1929) reported the distended condition of the uterine cornua of the mouse during prooestrus and oestrus. Marco (1930) demonstrated the presence of a considerable quantity of fluid in the uterine horns of the dog, but the uterus is not distended to the degree found in the rat and mouse. Evans (1933), however, was not impressed by the amount of fluid present in the uterus of the dog. Brambell and Rowlands (1935), in their study of the reproductive organs of the bank vole, mention the collection of fluid in the uterus.

It is probable that some secretion does accumulate in the uterus of most mammals but not in very large quantities. Long and Evans (1922) found that the fluid in the uterus of the rat was released rather suddenly through the vagina late in the oestrous period. This was detected by determining the degree of dryness of the vagina at 3-hour intervals. They considered that the time when the uterine fluid was expelled coincided with the sudden appearance of fluid and leucocytes in the previously dry vagina and that these were "most probably of uterine origin."

The work reported here was undertaken to determine the exact amounts of fluid present in the uterus of the rat at various stages of the oestrous cycle and to ascertain its possible function. The author wishes to express his sincere thanks to Drs. H. L. Wieman and C. K. Weichert for their guidance and kind suggestions.

EXPERIMENTAL. *Series I. Amount of uterine fluid in uterus during each stage of oestrous cycle.* Adult female rats that had been having regular

oestrous cycles, as determined by daily vaginal smears, were weighed and killed at various periods of the cycle. The reproductive organs, considered here to be the ovaries, tubes, and uteri, were removed intact and as much extraneous tissue as possible dissected off under conditions which prevented any loss of moisture. The uteri and vaginae were separated at a point just posterior to the cervices. The ovaries, tubes, and uteri, with the contained fluid, were then weighed in closed weighing bottles. The tubal end of each uterine horn was then slit open and the fluid very gently expressed. The organs were then weighed again.

Each of the data presented in table 1 is the average weight in grams of determinations on eight animals for each stage of the oestrous cycle.

The figures show that the amount of uterine fluid present in the uterus at prooestrus is about 18 times greater than that in the dioestrous period. This increase which occurs within a 24 hour period is coincident with the growth of the follicles and the period during which the female will accept the male in copulation. There is also an increase in the weight of the

TABLE 1

| STAGE OF OESTROUS CYCLE | BODY WEIGHT | ORGANS + FLUID | ORGANS − FLUID | FLUID |
|---|---|---|---|---|
| Dioestrus | 280.0 | 0.6879 | 0.6621 | 0.0258 |
| Prooestrus | 289.2 | 1.4877 | 0.9878 | 0.4995 |
| Early oestrus | 293.7 | 1.4983 | 0.9849 | 0.5131 |
| Late oestrus | 279.6 | 1.5022 | 1.0130 | 0.4892 |
| Post oestrus | 268.7 | 0.6352 | 0.6103 | 0.0249 |

reproductive organs additional to the increase caused by the accumulation of uterine fluid. This is probably due to the presence of large maturing follicles in the ovary and the increased vascularity of the uterus.

*Series II. Effect of retention of uterine fluid on oestrous cycle.* Six adult female rats having regular oestrous cycles were used in this experiment. The animals were anesthetized and the posterior portion of the uterus and vagina exposed by a mid-ventral incision. The vagina was ligated very tightly just posterior to the cervices with surgical silk. It was found necessary to separate the urethra from the uterus for a short distance in order to tie the ligature in the proper place. Care was exercised not to tie off any of the uterine blood vessels during the operation. Three animals were operated in the dioestrous stage and three in the prooestrous stage of the cycle.

The animals were sacrificed on an average of 21 days after the operation and the reproductive organs removed. The uterine horns of all animals were found to be enormously distended by the collection of fluid irrespective of the stage of the cycle when the original operation was per

formed. This fluid was carefully removed by means of a 10 cc. hypodermic syringe and measured. The average total amount in both uterine horns was 5.5 cc. per animal.

After the initial operation, one of the animals showed a prolongation of each subsequent oestrous period of from 1 to 2 days throughout four cycles. One animal had a 12-day period of dioestrus immediately following the operation. This was probably a pseudopregnant period induced by stimulation of the cervices during the operation. The remaining animals did not show any alteration of the normal cycle.

*Series III. Effect of removal of uterine fluid on oestrous cycle.* Two types of procedure were employed in this experiment. In the first, 4 normal animals in prooestrus and early oestrus were anesthetized and the uterine fluid removed with a hypodermic syringe during each of six consecutive oestrous cycles. The amount of fluid removed each time measured between 0.4 and 0.5 cc., indicating almost complete removal. The second approach was to anesthetize the animals in prooestrus and remove a portion of the ovarian capsule. This method was not so successful since the capsule regenerated and closed again in approximately 9 days. Repeated operations were therefore necessary to keep the capsule open. The active uterine contractions during the prooestrous and oestrous periods probably caused the fluid to be expelled into the abdominal cavity. The oestrous cycles of animals in these experiments showed no deviation from the normal.

*Series IV. Effect of injections of uterine fluid into castrate females.* Four adult females that had been ovariectomized 24 days previously were given subcutaneous injections of the collected uterine fluid. Each animal received 1 cc. at each 12-hour interval until a total of 10 cc. had been injected. Daily vaginal smears showed no variation from the normal castrate condition. Two days after the last injection the animals were sacrificed and the reproductive tracts, mammary tissue, and the pituitary glands were removed and fixed for sectioning. Histological examination showed that all of the tissues were typical of castrate animals.

*Series V. Effect of removal of uterine fluid on sperm transport.* Eight adult female rats in the prooestrous period were anesthetized and the fluid removed from the uterus. These animals were placed in cages with normal males as soon as possible and permitted to copulate. Constant observations were made to determine the exact time of a fertile copulation as determined by the presence of spermatozoa in the vagina or the formation of the copulation plug. Immediately after copulation the animals were killed by decapitation. The body cavity was opened quickly and the uterine horns clamped in two places so as to divide them into three approximately equal compartments. Each of the three parts of the two uterine cornua was then perfused with saline solution and examined for the presence of spermatozoa.

The animals were sacrificed at intervals from 1.5 to 4.5 minutes after copulation and the uterine horns clamped 75 seconds later. Large numbers of motile spermatozoa were found in the posterior third of the uterus in the animals examined 2 minutes and 45 seconds after copulation, but none were present in the middle or tubal regions. The animals examined 5 minutes and 45 seconds after copulation showed many spermatozoa in the posterior two-thirds of the uterine horns, but few in the tubal third. The other cases presented intermediate stages. The average time required for the spermatozoa to reach the periovarial space under these conditions is approximately 4 minutes.

It was deemed necessary to test the time of transport of spermatozoa in the normal animal. Four normal females were permitted to copulate with normal males. As soon as a fertile copulation was effected, the females were quickly decapitated and treated in the manner described above. It was found that in 1 minute and 30 seconds after a normal copulation the spermatozoa had reached the periovarial space. This demonstrates that the transport of spermatozoa in the uterus of the normal female rat is very rapid and confirms the work of Hartman and Ball (1930).

Experiments were carried on wherein the fluid was removed from one uterine horn in the prooestrous period while the other was permitted to remain undisturbed. Sperm transport was much slower in the horns from which the fluid was removed.

Discussion. It is well known that the uterus of the rat shows a periodic wave of growth followed by retrogression. Investigators have demonstrated that in all mammals the uterine reactions are similar in that this wave of growth coincides with the growth of the follicles which precedes maturation of the ovum and ovulation. According to E. Allen (1932), it seems probable that species differences may depend upon variations in intensity or duration of secretion of oestrone or upon different rates of growth of these tissues.

W. M. Allen (1931) made an intensive study of the histological changes in the endometrium of the rat during the normal oestrous cycle, pseudopregnancy, and pregnancy. The surface epithelium and glands show the most noticeable changes, but the underlying connective tissue is also affected. The secretion of the uterine fluid is not accompanied by any evidence of growth of the glands of the uterine cornua since no mitoses are present. During the period of greatest secretion the glands are distended, but during the dioestrous period they are collapsed. The fact that their enlargement is due to the effect of oestrone is shown by the shrunken state following ovariectomy, during pseudopregnancy and pregnancy, and by the fact that they respond rapidly to injections of oestrone.

The evidence presented in this paper confirms the findings of Long and Evans (1922) concerning the time of expulsion of the uterine fluid.

It will be noted that the amount of fluid in the uterus following late oestrus is greatly decreased. The retention of the fluid as a result of the ligation of the vagina lends further support to the view that the fluid is expelled through the vagina in late oestrus and not resorbed. Rossman (1937) failed to notice the expulsion of any fluid through the vagina during the course of his experiments. If the fluid were resorbed and not released, it would probably not accumulate in such large quantities in the ligated uterus.

The injection experiments which have been carried on seem to eliminate any possibility of hormonal action on the part of the uterine fluid. Removal of the fluid in consecutive cycles shows that it is not necessary for the maintenance of the normal cycle.

Previous investigators have suggested that aid in sperm transport is one function of the uterine fluid of the rat. Much work has been done concerning the mechanism of sperm transport in various animals. Whitney (1927) found spermatozoa in the ovarian bursa of the dog 20 minutes after the beginning of a copulation which lasted 18 minutes. Hartman (1923) showed that sperm transport in the opossum is rapid. Hartman and Ball (1930) performed a series of experiments on the transport of spermatozoa through the cervices and in the uterus of the rat. They found that animals killed 30 seconds after copulation and having the uterine horns clamped in the middle region 24 seconds later showed sperm in the posterior or lower half of each uterine horn but none in the anterior part. Their conclusion was that "the transport of sperms, at least in the uterus of the rat, is a matter not of hours, but of seconds." Parker (1930) found the rabbit uterus to be sluggish and estimated that 2 hours were required for sperm transport. Evans (1933) demonstrated by means of uterine fistulae that spermatozoa arrive in the oviducts of the dog 25 seconds after ejaculation of the male. Phillips and Andrews (1937) found that ram sperm required from 30 minutes to 7 hours and 7 minutes after insemination to traverse the entire length of the genital tract of the ewe.

The reports of these investigators show then that in animals where there is a collection of uterine fluid the sperm transport is rapid. In forms like the rabbit, where the amount of fluid is small, a longer period is required for the spermatozoa to travel through the uterus to the Fallopian tubes.

Florey and Walton (1932) prepared apical fistulae in the uterine horns of rats to permit the fluid to escape. Spermatozoa reached the Fallopian tubes just as rapidly as in normal rats. Although these workers fail to mention the amount of fluid in such uteri, their conclusion was that large amounts of fluid are unnecessary for sperm transport in the rat. Rossman

(1937) studied the contractions of the rat uterus in vivo and described two types. He reported that the movement of the uterine fluid due to these peristaltic contractions of the uterus could propel the sperm from the cervical end to the tubal end of the uterus in little more than a second's time. The speed of the tubally-directed currents set up by these contractions which move toward the vagina rule out antiperistalsis as the motive force in sperm transport in the uterus of the rat.

That the rapid transport of the spermatozoa in the rat depends on a sufficient quantity of fluid to establish the tubally-directed currents is evident. In experiments where the fluid is almost entirely removed, the transport is perceptibly slowed up. The disagreement with the interpretation of the work of Florey and Walton can only be explained on the basis of incomplete removal of the fluid through fistulae present in the apical ends of the uterine horns. In the absence of sufficient fluid, the sperms together with the product of the seminal vesicles would be piled up at the cervical end of the uterus by the contractions. The uterine fluid seems to be the necessary medium for the movement of the spermatozoa toward the periovarian space.

The chemical nature of the uterine fluid has not been determined in these experiments. Owing to the difficulty in collecting sufficient quantities, few attempts at analysis have been made.

## SUMMARY

1. The amount of uterine fluid present in the uterus of the rat in each stage of the oestrous cycle has been determined.

2. The observation of Long and Evans that the uterine fluid is expelled during late oestrus has been confirmed.

3. There is apparently no hormonal influence exerted by the uterine fluid upon the oestrous cycle or, when injected, upon the reproductive organs of castrate females.

4. The function of the uterine fluid of the rat seems to be that of serving as a medium for the transportation of spermatozoa.

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# THE EFFECTS OF UNILATERAL NEPHRECTOMY ON THE RENAL BLOOD FLOW AND OXYGEN CONSUMPTION OF UNANESTHETIZED DOGS[1]

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Received for publication February 10, 1938

Herrick, Essex and Baldes (1932), using the method of the Thermo-Stromuhr of Rein, found that removal of one kidney had no effect on the blood flow to the remaining kidney during the first three hours. Van Slyke, Rhoads, Hiller and Alving (1934), using unanesthetized dogs with an explanted kidney, calculated the renal blood flow by determining the rate of urea excretion and the decrease in blood urea content which occurred as the blood passed through the kidneys. The oxygen content of arterial blood and renal venous blood were determined and the oxygen consumption was calculated. They state, "Removal of one kidney was followed by an increase in the blood flow, oxygen consumption, and urea clearance of the remaining kidney. The increase in most cases appeared to reach its maximum within a month. The average increase in blood flow was 68 per cent, in oxygen consumption 81 per cent, and in urea clearance 43 per cent, of the pre-operative values." These authors enumerate the results of other observers who have determined the rate of blood flow through one kidney of the dog, and state that their studies are the first which were performed without operation or anesthesia.

A method was described recently by Mason, Blalock and Harrison (1937) which enables one to determine directly the flow of blood through the kidneys of unanesthetized dogs and which also permits one to obtain renal venous blood for analysis. The present publication deals with the results of the application of this method to a study of the effects of unilateral nephrectomy on the renal blood flow and oxygen consumption as determined at varying intervals following the removal of the kidney.

METHOD. Mature dogs of unknown ages and weighing approximately 12 kgm. were used. They were trained to lie quietly upon the table. Their diet consisted of a standard kennel ration supplemented by meat three times each week. Food was withheld the 20 hours preceding the

[1] Aided by a grant to Vanderbilt University from the Division of Medical Sciences of the Rockefeller Foundation.

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studies. No anesthetic was used except for the injection of novocain over the external jugular vein at the site where a cannula was to be introduced. The animals exhibited no evidence of pain during the course of the experiments. The principle of the method for measuring the blood flow consists of producing a temporary blockage of the inferior vena cava above and below the entrances of the renal veins, and of diverting the renal blood during this brief period into a cannula which had been passed into the inferior vena cava through the external jugular vein. The temporary blockage was produced by inflating two balloons which were attached to the cannula. Small veins which empty into the inferior cava in the neighborhood of the renal veins were ligated at a preliminary operation. After determining the rate of renal flow, the sample of renal venous blood for analysis was withdrawn from the cannula and the arterial blood was obtained from the femoral artery. Heparin was used as the anticoagulant.

Approximately one week after having determined the total renal blood flow and oxygen consumption of the two kidneys, the left kidney was removed under ether anesthesia and weighed. After intervals varying from 3 to 92 days, the determinations were repeated on the remaining kidney. In a few instances, a third series of studies was performed, the longest time separating the nephrectomy and the last study being 118 days. Following the completion of the experiment, the animal was anesthetized, the position of the cannula was checked and the remaining kidney was removed and weighed.

Results. Twelve experiments were performed. The shortest interval of time separating the nephrectomy and the ensuing studies was three days and the longest interval was 118 days. Assuming the total weight of the two kidneys to be twice that of the left which was removed, nephrectomy was followed by an increase in blood flow per gram of kidney tissue in all instances. This increase apparently begins very shortly following the nephrectomy, is somewhat more rapid during the first month than subsequently but continues for approximately three months. At the end of this time, the flow in most instances was approximately the same as it had been through the two kidneys. If it be assumed that the two kidneys weighed the same at the beginning of the experiment, there was in most instances a definite increase in the weight of the remaining kidney but the percentage increase in flow was considerably greater than the percentage increase in weight. The results of all experiments are given in table 1. The relationship of the increase in flow to the interval following nephrectomy is shown in graph 1.

The renal arteriovenous difference in oxygen content remained about the same in seven experiments and decreased somewhat in four. These determinations are subject to more error than the blood flow measure-

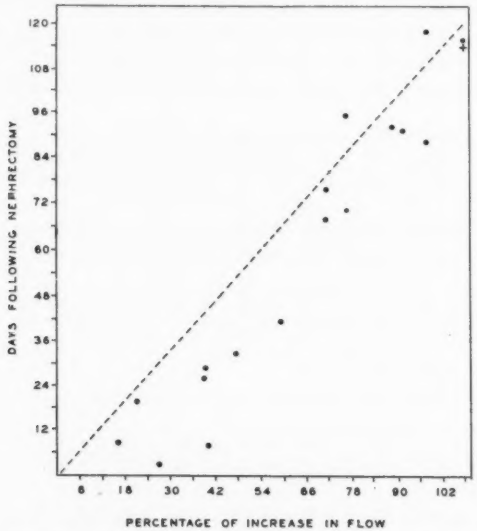
TABLE 1

*The effects of unilateral nephrectomy on the renal blood flow and oxygen consumption*

| DOG NUMBER | TIME CONTROL AND DAYS AFTER NEPHRECTOMY | WEIGHT DOG | RENAL BLOOD FLOW | | OXYGEN CONTENT | | A.V. DIF. $O_2$ | $O_2$ CONSUMPTION | | WEIGHT LEFT KIDNEY | WEIGHT RIGHT KIDNEY |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Total | Per gram kidney* | Arterial | Venous | | Kidneys or kidney | Per gram kidney* | | |
| | | *kgm.* | *cc. per min.* | *cc. per min.* | *vols. per cent* | *vols. per cent* | | *cc. per min.* | *cc. per min.* | *grams* | *grams* |
| 1 | Control | 13.7 | 246 | 3.28 | 18.60 | 17.18 | 1.42 | 3.49 | 0.047 | 37.5 | |
| | 68 | 12.7 | 210 | | 17.52 | 15.62 | 1.90 | 3.99 | | | |
| | 118 | 14.6 | 244 | 6.10 | 20.01 | 18.36 | 1.65 | 4.03 | 0.101 | | 40.0 |
| 2 | Control | 13.5 | 270 | 3.51 | 16.49 | 14.04 | 2.45 | 6.61 | 0.086 | 38.5 | |
| | 76 | 14.3 | 231 | | 18.24 | 15.22 | 3.02 | 6.98 | | | |
| | 116 | 13.0 | 294 | 5.3 | 18.10 | 15.50 | 2.60 | 7.64 | 0.138 | | 55.5 |
| 3 | Control | 11.9 | 273 | 4.0 | 20.72 | 16.90 | 3.82 | 10.43 | 0.154 | 34.0 | |
| | 41 | 11.2 | 217 | | 19.77 | 17.87 | 1.90 | 4.12 | | | |
| | 96 | 13.1 | 240 | 6.1 | 18.11 | 16.83 | 1.28 | 3.07 | 0.078 | | 39.6 |
| 4 | Control | 13.2 | 174 | 1.91 | 15.20 | 12.13 | 3.07 | 5.34 | 0.059 | 45.5 | |
| | 92 | 14.6 | 164 | 3.17 | 16.49 | 13.36 | 3.13 | 5.13 | 0.099 | | 51.7 |
| 5 | Control | 11.6 | 214 | 3.77 | 21.49 | 18.38 | 3.11 | 6.65 | 0.117 | 28.4 | |
| | 91 | 9.0 | 205 | 4.73 | 11.50 | 8.05 | 3.45 | 7.07 | 0.163 | | 43.3 |
| 6 | Control | 13.9 | 303 | 3.85 | | | | | | 39.3 | |
| | 88 | 13.7 | 300 | 6.15 | | | | | | | 48.8 |
| 7 | Control | 11.3 | 167 | 2.41 | 15.65 | 11.82 | 3.83 | 6.39 | 0.092 | 34.7 | |
| | 33 | 10.0 | 123 | 3.73 | 16.81 | 14.34 | 2.47 | 3.40 | 0.103 | | 33.0 |
| 8 | Control | 12.4 | 176 | 3.46 | 16.53 | 13.23 | 3.30 | 5.81 | 0.114 | 25.4 | |
| | 26 | 11.9 | 122 | 4.24 | 14.29 | 11.31 | 2.98 | 3.64 | 0.126 | | 28.8 |
| 9 | Control | 12.4 | 265 | 4.45 | 24.90 | 20.18 | 4.72 | 12.50 | 0.210 | 29.8 | |
| | 20 | 13.2 | 160 | 4.89 | 23.40 | 21.09 | 2.31 | 3.70 | 0.113 | | 32.7 |
| 10 | Control | 12.9 | 326 | 4.29 | 15.40 | 12.60 | 2.80 | 9.13 | 0.120 | 38.0 | |
| | 16 | 12.2 | 177 | | 14.97 | 12.09 | 2.88 | 5.10 | | | |
| | 29 | 11.6 | 226 | 5.86 | 13.17 | 10.73 | 2.44 | 5.52 | 0.143 | | 38.6 |
| 11 | Control | 14.0 | 227 | 2.73 | 13.52 | 10.08 | 3.44 | 7.81 | 0.094 | 41.6 | |
| | 3 | 13.8 | 144 | 3.46 | 12.79 | 9.58 | 3.21 | 4.62 | 0.111 | | 41.6 |
| 12 | Control | 12.3 | 303 | 4.07 | 16.46 | 13.46 | 3.00 | 9.09 | 0.122 | 37.2 | |
| | 8 | 11.6 | 212 | 5.30 | 10.96 | 7.72 | 3.24 | 6.86 | 0.172 | | 40.0 |

* The control studies on blood flow and oxygen consumption per gram of kidney are figured on the basis of doubling the weight of the left kidney.

ments because the kidney utilizes a smaller proportion of the oxygen in the blood than do most of the other tissues in its neighborhood and a small error in a blood flow determination is apt to be associated with larger errors in determining the arteriovenous oxygen difference and oxygen consumption. At any rate nephrectomy was in most instances followed by very little alteration in the renal arteriovenous difference in oxygen content. Since this is true, the changes in oxygen consumption following nephrectomy bore a fairly direct relationship to the alterations in the renal blood flow. An increase in oxygen consumption per gram of kidney tissue was found following the removal of one kidney in all experiments




Graph 1. Shows the percentage increase in flow in relationship to the days following nephrectomy. Renal blood flow increased somewhat more rapidly during the first month than subsequently but the increase continued in most instances for three months or longer.

with two exceptions. We doubt the correctness of these latter experiments. These results are given also in table 1.

Discussion. The results agree in the main with those of Van Slyke, Rhoads, Hiller and Alving (1934) except that we found a somewhat slower increase in renal blood flow and oxygen consumption. The increase in most of their experiments appeared to reach its maximum within a month, whereas in our studies, it required usually about three months. Furthermore, Van Slyke et al. found an average increase in flow in the remaining kidney of 68 per cent, while the increase in our experiments in which the animals were followed for three months or longer was usually greater than 90 per cent.

Regarding the oxygen consumption, Van Slyke et al. state, "Our data agree with those of previous authors in indicating that the oxygen consumption varies within wide limits." In our experiments, the oxygen consumption increased approximately as much as the renal blood flow and wide variations occurred in only two experiments, the accuracy of which we doubt.

Observations on renal blood flow are of interest in that the evidence indicates that the flow is a good index of the functional activity of the kidneys. Medes and Herrick (1933) found that the creatinine clearance parallels the renal blood flow as measured by the Stromuhr. Van Slyke et al. (1934) state that change in renal blood flow in the normal dog is the main physiological factor which determines alterations in urea clearance. Addis, Myers and Oliver (1924) performed unilateral nephrectomy on rabbits and noted that the average urea clearance was 63 per cent of its preoperative value 15 to 33 days following operation. Approximately four months after the nephrectomy, the average value equalled 79 per cent of the preoperative one and some hypertrophy of the remaining kidney was observed. Karsner, Hanzal and Moore (1934) found a decrease in urea clearance in dogs shortly following nephrectomy and a return to essentially the control level in six months. The remaining kidney increased in weight by from four to 58 per cent as compared with the kidney that was removed. Ellis and Weiss (1933) have found in man the urea clearance by a single normal kidney following nephrectomy to be within the range 70 to 127 per cent of mean normal.

## SUMMARY

The effects of unilateral nephrectomy on the renal blood flow and oxygen consumption have been determined in unanesthetized dogs. Removal of one kidney is followed by a slowly progressive increase in the blood flow of the remaining kidney, somewhat more rapid in the first month than subsequently, and reaching approximately the combined flow of the two kidneys at the end of three months. Nephrectomy is associated usually with very little alteration in the renal arteriovenous difference in oxygen content. Alterations in renal oxygen consumption usually parallel changes in renal blood flow.

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# ELECTRIC STIMULATION AND CONDUCTION OF EXCITATION IN SMOOTH MUSCLE

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Received for publication January 10, 1938

The muscular coat of the viscera contains an enormous number of fibers. Uncoördinated activity of the small muscle cells, therefore, could never produce the regular movements which are observed in these organs. In the absence of external stimuli rhythmic contractions of smooth muscles can only be understood by postulating some mechanism of conduction which coördinates the activity of the numerous elements. At present it is generally assumed that this function is performed by a diffuse ganglionic plexus, present in the musculature of most viscera. However, this explanation is not applicable in the case of uterine musculature, which is free of ganglion cells and is capable of coördinated activity. A simple explanation of these facts was found in the assumption that the musculature of the uterus is a syncytium like cardiac muscle and physiological evidence for this assumption will be presented in this paper.

The results which were obtained for uterine muscle confirm a large part of the remarkable work of Engelmann (1869, 1870) on the ureter. This author observed that mechanical and electrical stimulation of the ureter produced a contraction wave which was propagated in either direction. Because Engelmann was unable to demonstrate ganglion cells in the ureter, except at both extreme ends, he concluded that the propagation of excitation was entirely muscular and that the muscular tissue was a syncytium. This conclusion was supported particularly by the observation that electric current had the same polar effects as in nerve. Galvanic current initiated the response at the cathode and produced electrotonic changes.

Engelmann's conclusions were not generally accepted because ganglion cells were observed in the adventitia of the ureter over its whole length. It seemed possible, therefore, to explain the properties of this organ on the assumption that excitation was conducted by nervous elements. No decisive arguments for or against Engelmann's views have been advanced more recently (see Gruber, 1933, for references), however, some facts, which will be mentioned later, make it reasonably certain that nervous elements are not essential for the responses of the ureter.

The electric excitability of smooth muscle was studied by many investigators. Usually no attempt was made to decide whether the responses were due to direct stimulation, but Monnier succeeded in eliciting contractions of the denervated nictitating membrane of the cat by electric stimulation. Extensive studies of the responses of the latter muscle, produced by indirect stimulation, were made by Eccles and collaborators (Eccles, 1936, for references), who obtained indirect evidence for the existence of a refractory phase and the validity of the all or none relation. On the other hand, Cannon and Rosenblueth (1937), on the basis of experiments on the uterus of the cat, arrived at the conclusion that the responses of smooth muscle produced by electric stimulation are due to stimulation of motor nerves or due to the injurious effect of the strong electric currents used.

In the present paper it will be shown that visceral smooth muscle can be stimulated directly by electric current and that the excitability of this type of muscle differs only quantitatively from nerve and striated muscle, as indicated by measurements of the strength duration relationship, the rate of conduction and the refractory phase.

MATERIAL AND METHOD. The uterus and ureter of guinea pigs, rabbits and cats were used. The uterus was cut longitudinally into strips, about 1 mm. wide. The mucous membrane and the underlying layer of muscular tissue were removed. Only exceptionally excitable uterine preparations showed spontaneous movements.

The muscles were immersed in Ringer's solution, buffered by 0.01 per cent $Na_2HPO_4$. Zinc-zinc sulphate brush electrodes, which made contact with the muscle by cotton pads soaked in saline, were used for stimulation. The preparation, mounted on a glass plate, was enclosed in a moist chamber which was immersed in a constant temperature bath (32°–38°C.). Solutions could be applied to the preparation through a glass tube. Galvanic current or condenser discharges (7$\mu$F) were suitable stimuli. For the measurement of chronaxie, rectangular shocks, obtained by a revolving contact breaker, were used. The rate of conduction was roughly estimated by following the spread of the active region of a muscle. In some experiments the mechanical responses were recorded by a sensitive isometric lever.

The ureter is more suitable for quantitative studies than uterine strips. Observations on this organ will be reported here in as far as they supplement the results from uterine strips.

RESULTS. In excitable uterine strips a weak electric field (0.2 to 0.4 volt) produces a response which is propagated over the whole preparation. Excitability and the ability to conduct vary and depend on hormonal influences. During anestrus, strips of the cat's uterus do not give any visible response to electric stimuli, however strong. A propagated re-

sponse, however, can be obtained from uterine strips of guinea pigs and rabbits during estrus and from cats if intraperitoneal injections of theelin have been given previously (200 int. units, 3 times daily for 4 or more days). Such preparations were used for the experiments described below. Also the uterus of pregnant guinea pigs is excitable electrically.

Adrenaline makes uterine strips from cats non-excitable whereas cocaine in low concentrations sensitizes both the ureter and the uterus of all the species tested.

1. *Characteristics of excitability.* Chronaxie, rate of conduction and the duration of the refractory phase were determined; the average values found are summarized in table 1. The most remarkable finding is the great and consistent quantitative difference between different smooth muscles. The time factor involved is long in all the muscles tested. In uterine strips from guinea pigs the rate of conduction was sometimes less than 1 mm. per second. The rates of conduction are clearly correlated

TABLE 1

*Characteristics of excitability of some smooth muscles*

| | CHRONAXIE | RATE OF CONDUCTION | ABSOLUTELY REFRACTORY PHASE |
|---|---|---|---|
| | *m. sec.* | *mm. sec.* | *sec.* |
| Uterus of guinea pig 32°–36° | 300 700 | 1–3 | <1 |
| Uterus of rabbit 35° | 200 | 10 | <1 |
| Uterus of cat 35° | 70 | 60 | <1 |
| Ureter of rabbit 35° | 180 | 20 | 3 |
| Ureter of cat 35° | 50 | 50 | 1.5 |

with the chronaxies. Both the uterus and the ureter are fastest in the cat and slowest in the guinea pig.

Conduction is not interrupted by cutting the uterus in the shape of a zigzag band. This shows that conduction proceeds not only along longitudinal muscle strands but also transversely.

In visceral muscle conduction may result from the mechanical stimulation, by an active region, of adjacent parts of the muscle, as shown by the observations of Cannon (1912), Trendelenburg (1917) and Alvarez (1929) for the peristaltic waves in the gastro-intestinal tract. It seems improbable that conduction of this type is involved in the experiments reported here because muscle preparations which were so slack that the mechanical response was barely visible, were able to conduct, whereas only a strong stretch elicited a contraction.

Figure 1 is a strength duration curve obtained from the ureter of a rabbit.

Figure 2 represents the recovery of excitability following a response

in the rabbit's ureter. Very weak contractions could be obtained by strong shocks about one second after an effective stimulus. However during the first three seconds the responses were not propagated and were limited to the region of the cathode. Since the criterion of the absolutely refractory phase is the absence of a conducted response, the duration of this phase has to be taken roughly as 3 seconds. It is noteworthy that, even during this period, local responses could be elicitied by strong stimuli.

Because of the long duration of the refractory state it is impossible to tetanize the ureter (fig. 3). The refractory period of uterine muscle, on the contrary, is short compared with the mechanical response; uterine strips, therefore, can be tetanized (fig. 4).

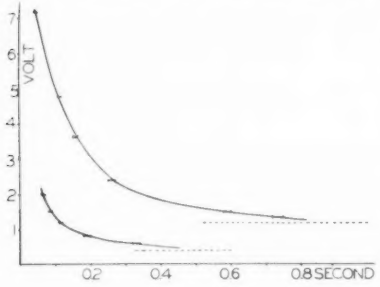


Fig. 1

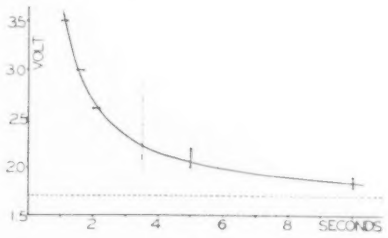


Fig. 2

Fig. 1. Rabbit's ureter. Two strength duration curves from different preparations. Rectangular shocks. Abscissa: duration. Ordinate: minimal voltage. Interrupted lines: rheobases.

Fig. 2. Rabbit's ureter. Recovery of excitability during the refractory period. Conditioning stimulus a rectangular shock of 0.05 sec. duration; test shock a condenser discharge. Ordinates represent the minimal voltage of the test shock; abscissae, interval between test shock and conditioning shock. Interrupted line indicates the normal threshold of test shock. Dotted line: smallest interval after which conduction takes pace, irrespective of the strength of the stimuli.

2. *Can smooth muscle be stimulated directly?* The possibility that the responses of the uterus and ureter are caused by the stimulation of motor nerve fibers can be excluded for several reasons.

*a.* Stimulation of the nerves supplying the uterus of the cat and guinea pig causes only inhibition, showing the absence of motor fibers (Gruber, 1933, for references).

*b.* Cocaine in concentrations far in excess of those necessary to block all nervous conduction interferes neither with the excitability nor with the propagation of the response. Cocaine 1:200 produces only a weakening of the mechanical response. Cocaine 1:100 blocks conduction.

*c.* The characteristics of excitability, chronaxie, rate of conduction and the duration of the refractory period have a magnitude different from

that of even the slowest nerve fibers. This argument by itself does not entirely disprove the possibility that motor nerve fibers are involved, because it may be assumed (Cannon and Rosenblueth, 1937) that the properties of the thin nerve fibers inside the smooth muscle tissue differ from those of the fibers in nerve trunks.

*d*. The possibility that the excitatory phenomena described above are due to the so-called interstitial cells must be discussed. These cells, (Stoehr, 1932, for references) form a diffuse syncytial plexus within the muscular coat of viscera. They are considered as nervous elements by some authors and as connective tissue cells by others. Tiegs (1925) and van Esveld (1928) have suggested that the automatic movements of the intestine may originate in these cells.

It can be shown, however, that a diffuse plexus of any kind is not involved in the responses of uterine muscle. Whereas striated muscle and nerve do not respond to currents passing at right angles to their fibers,

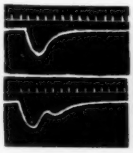

Fig. 3

Fig. 4

Fig. 3. Responses of the ureter of the cat; upper graph: response to single shock; lower graph: response to shock followed by second shock applied during the refractory period. Temp. 35°. Isometric recording. 1 g. = 1 cm. Time marks every second.

Fig. 4. Temp. 34°. Response to single shock and tetanus of uterine strip of cat which has previously received injections of theelin. Isometric recording. 1 cm. = 1 g. Temp. 35°. Time marks every second.

a diffuse nerve plexus would be stimulated equally well by electric currents having different orientation with respect to the muscle fibers. This alternative was tested, using uterine strips from guinea pigs, about 5 mm. wide. The rheobase was determined, first, when the current passed longitudinally, the distance of the electrodes being equal to the width of the strip. After rearranging the cotton pads serving as stimulating electrodes so that the current passed at right angles to the strip, the rheobase was determined again. It was, then, 10 to 20 times higher than before.

The effect of orientation of the stimulating current could be shown even more strikingly by two slight modifications of the experiment just described. Conduction was impaired by low temperature (30°–32°C.), so that a contraction of the circular muscle fibers could be obtained without any response of the longitudinal fibers. Furthermore, strips from pregnant animals were used because the tissue of the uterine wall becomes looser during pregnancy, making it easier to distinguish and separate

individual strands of longitudinal and circular muscle fibers. Under these conditions, electric current passing at right angles to the preparation stimulated only the strands of circular fibers, whereas the longitudinal fibers contracted when the current passed longitudinally.

In one particular experiment the rheobase for the longitudinal muscle fibers was 350 m. volt (electrode distance 5 mm.) if the current passed longitudinally. After rearranging the electrodes so that the current passed at right angles to the strip, the strands of circular fibers alone responded at 1.8 volt, whereas the longitudinal fibers did not respond at 9 volt. The electrodes were finally rearranged for stimulation in longitudinal direction and a rheobase of 430 m. volt was found.

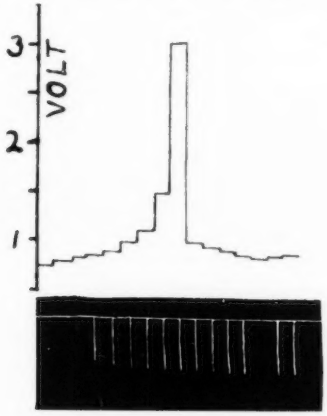


Fig. 5. Responses of a strip from the cat's uterus, demonstrating all or none relation. Stimulation by rectangular shocks of 0.3 sec. duration. Voltage applied indicated in upper graph. Isometric recording. Tension produced in each response about 1 g. Temp. 33°.

3. *Evidence for the syncytial character of smooth muscle.* If it is accepted that excitation of smooth muscle is purely muscular, it must be assumed, also, that conduction is muscular and not due to any nervous elements within the tissue. Since smooth muscle fibers are very short, it must be postulated, then, that excitation can be transmitted from muscle cell to muscle cell as in cardiac muscle.

This conclusion is supported by several additional observations, indicating that the muscle preparations used are single muscular units, namely, *a*, the validity of the all or none relation; *b*, the polar effects of the electric current; *c*, the absolute value of the rheobase, which has the same order of magnitude as in nerves and muscles consisting of long fibers; *d*, the existence of injury potentials and action potentials.

*a*. Figure 5 shows for the cat's uterus that every effective stimulus

produces a maximal response. Similar records were obtained from the uterus of the guinea pig and the ureter of the cat.

As shown by studies of action potentials (unpublished), single stimuli, under certain conditions, produce a repetitive discharge. The all-or-none law seems to hold also for these tetanic contractions. Uterine strips from cats which had received injections of theelin for 4 days were mainly used for the experiments just described. They rarely gave repetitive responses.

*b.* If the single smooth muscle fibers were independent units, all the fibers between the electrodes should behave alike, since all are under the influence of the same electric field. The whole muscle, consisting of innumerable small cells, should, therefore, contract or relax simultaneously under the influence of an electric current and there could only be a difference of the response between both ends of each muscle cell.

Actually, however, the smooth muscle preparations used react to an electric current as if they were single cells. Engelmann (1870) observed that the responses of the ureter on making a galvanic current start at the cathode. The same is true for uterine strips.

*c.* If the distance between the stimulating electrodes was 4 cm., the rheobase usually was below 0.4 volt. Since the single fibers are only about 0.05 mm. long the potential difference applied to one cell during an effective stimulus was less than 0.5 m. volt, many times less than the minimal voltage for the fibers of nerves and of striated muscle. This fact is intelligible if the muscles studied are considered as the equivalent of single large cells, like cardiac muscle.

The rheobase of some other smooth muscles, like the denervated nictitating membrane (Monnier, 1936), is very much higher than that of the muscles used here. This difference is not unexpected in view of the short length of the muscular units of the nictitating membrane.

*d.* Injury potentials should not be expected to occur in muscles consisting of units as short as single smooth muscle cells because these units would presumably be killed as a whole and the dead cells would merely serve as a lead for the intact portion of the muscle. Injury potentials, however, have been observed by Engelmann (1877) on the frog's stomach, Bacq and Monnier (1935) on the nictitating membrane, the uterus and urinary bladder of the cat. The potential differences observed are rather small (as much as 8 m. volt in the frog's stomach) compared with those of striated muscle, but this is not unexpected in view of the great amount of connective tissue present in most smooth muscles.

In my own experiments injury potentials were detected consistently after applying 1 per cent KCl on an intact portion of a muscle preparation and were found 1.5 to 2.0 m. volt in the guinea pig's ureter and 2.4 to 4.4 m. volt in strips from the cat's uterus.

Also the existence of action potentials in smooth muscle is difficult to

reconcile with the assumption that the single cells are units comparable to striated muscle fibers because the potential differences produced by the innumerable cells lying between two electrodes should be expected to cancel out. Sufficiently large potential differences could be produced if the electric changes were asymmetrical in all the cells in a manner suggested by Rosenblueth, Davis and Rempel (1936). Such an assumption becomes unnecessary, at least for visceral muscles, by considering smooth muscle as a syncytium.

DISCUSSION. It cannot be decided as yet whether the results obtained for the uterus and ureter can be generalized to include other kinds of smooth muscle. It does not seem probable that widespread syncytial connections occur in smooth muscles supplied by true motor nerves, like the musculature of blood vessels or the nictitating membrane. It is noteworthy, however, that injury potentials (Bacq and Monnier, 1935) and action potentials (Cannon and Rosenblueth, 1937, for references),

TABLE 2

*Characteristics of excitability of smooth muscle as compared with nerve*

| | VELOCITY | CHRONAXIE | ABSOLUTELY REFRACTORY PHASE |
|---|---|---|---|
| | *cm. sec.* | *m. sec.* | *m. sec.* |
| Frog A-fibers, 20°* | 2500 | 0.3 | 1 |
| Frog C-fibers, 20°* | 100 | 7 | 20 |
| Cat's ureter, 35° | 6 | 50 | |
| Rabbit's ureter, 35° | 2 | 200 | 3000 |
| Guinea pig's uterus, 35° | 0.3 | 600 | |

* Erlanger from Erlanger and Gasser (1937).

have been observed in the latter organ, suggesting that the physiological units are larger than single muscle cells.

The conclusion that visceral muscles behave like syncytia and that excitation can be conducted from cell to cell is supported by studies of the structure of visceral smooth muscle (Häggquist, 1931, and Boeke, 1932, for references), indicating that there are broad anastomoses between the muscle cells. There is consequently a perfect analogy between cardiac and visceral muscle, both histologically and physiologically.

It may seem difficult to understand on this basis the great variety of movements of viscera. However, various types of motility may be the result of quantitative differences of the excitability of different smooth muscles. For instance, the different duration of the refractory phase of the uterus and ureter, or the different velocity of conduction in the uterus of different species must produce a different type of activity. Furthermore, excitability and conduction are influenced by hormones, as shown

by the effect of theelin and adrenaline on the cat's uterus, and possibly also by nervous action.

It seems interesting to compare some of the properties of smooth muscle with those of other excitable tissues. As shown in table 2, the difference between the fastest and slowest nerve fibers is greater than that between slow nerve fibers and some smooth muscles. It seems probable, therefore, that the differences between these types of tissues, as far as excitatory phenomena are concerned, are only quantitative.

## SUMMARY

The responses of two visceral smooth muscles, the uterus and ureter, to electric stimuli were studied. Uterine strips were used for most experiments because they do not contain a ganglionic plexus.

In suitable uterine preparations weak electric stimuli produce a contraction which is conducted over the whole muscle in either direction at a slow velocity.

The electric excitability of uterine muscle is greatest during estrus. During anestrus the uterus is entirely inexcitable (as in the cat) or it gives only weak contractions which are not propagated (as in guinea pig). The excitability is increased by injections of theelin (p. 615).

Several facts disprove the assumption that the responses are due to stimulation of motor nerves. *a*. Only inhibitory nerves are present in the uterus of some of the species used like the cat; *b*, cocaine 1:200 does not abolish excitability or conduction; *c*, chronaxie and refractory phase are much longer than in the slowest type of nerve fibers (p. 617); *d*, muscle fibers oriented at right angles to the direction of flow of an electric current are not stimulated, proving that a diffuse nervous plexus is not responsible for excitation (p. 618).

Since the excitatory phenomena of smooth muscle are not due to nervous structures it must be assumed that excitation can be conducted from muscle cell to muscle cell. The muscles studied in this paper, therefore, must be considered as syncytia. This conclusion is confirmed by some further experimental results which indicate a close agreement between the properties of visceral smooth muscle and cardiac muscle. *a*. The all or none relation is valid (p. 619); *b*, the electric current has polar effects as in nerve and muscles consisting of long fibers (p. 620); *c*, the rheobase has the same order of magnitude as in muscles consisting of long fibers (p. 620); *d*, injury potentials and action potentials are present.

A comparison of the properties of visceral smooth muscle with those of nerve indicates that the differences between these tissues, as far as excitatory processes are concerned, are purely quantitative (p. 622).

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# DURATION OF SENSITIVITY OF THE ENDOMETRIUM DURING LACTATION IN THE RAT

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Received for publication February 12, 1938

Sensitization of the endometrium such that deciduomata can be experimentally produced following mechanical irritation is now well established as a function of the corpus luteum. These decidual tumors, however, cannot be produced at all times when the corpora lutea are known to be functional, but only at approximately the period when implantation normally occurs. Even during pregnancy the sensitization lasts for only a short time (Courrier and Kehl, 1930, rabbit; Allen, 1931, rat) despite the fact that in both these species the corpora are necessary practically to term. It is obvious that, while the ability of the uterus to produce deciduomata may be taken as evidence of functional activity of the corpora, the inability to produce deciduomata can not be considered as proof that the corpora are not functional.

There is one condition, however, in which the period of sensitization appears to be prolonged. During lactation in the rat Long and Evans (1922) found the uterus sensitive from the fourth to the tenth day, with occasional positive results still later, and Selye and McKeown (1935) found sensitivity to the 13th day. It is, of course, well established that the endometrium is sensitized in the early days of lactation in much the same manner as during pseudopregnancy and pregnancy, because ovulation in this species occurs spontaneously within twenty-four hours of parturition and the corpora which result are functional. Neither group of animals recorded by the above workers was large enough to make it feasible to conclude more than that the sensitivity persists longer during lactation than during pregnancy; they are not adequate to define the period when sensitivity becomes much less or disappears. This point seems to us to be sufficiently important to warrant study of a larger group of animals, especially since other experiments being carried out during the anoestrus of lactation require for their interpretation a better understanding of the standard conditions of sensitivity during this period.

The study which we have made to augment the existing data was carried out in mature albino rats. As soon as the presence of pregnancy

could be determined by palpation, the pregnant animals were removed from the breeding cages to individual cages and the exact day of parturition recorded. The parturient females were then permitted to nurse the entire litter throughout the period of experiment. A series of animals was studied in which, at various times during lactation, under ether anesthesia the right horn of the uterus was mechanically stimulated by multiple perforations with a needle, care being taken to assure injury to the endometrium. The series includes animals stimulated from the first to the 19th postpartum day inclusive. Five days following operation the animals were killed, the presence or absence of deciduomata noted, and the reproductive tract fixed in Bouin's fluid. The presence of deciduomata was then confirmed in all cases by microscopic examination of the

TABLE 1

*Production of deciduomata during lactation in the rat*

| DAY (POSTPARTUM) OF STIMULATION | NUMBER OF ANIMALS | NUMBER WITH DECIDUOMATA 5 DAYS LATER | PER CENT POSITIVE |
|---|---|---|---|
| 1 | 3 | 0 | 0 |
| 2 | 3 | 0 | 0 |
| 3 | 5 | 0 | 0 |
| 4 | 7 | 5 | 71.4 |
| 6 | 4 | 4 | 100 |
| 8 | 5 | 5 | 100 |
| 10 | 4 | 3 | 75 |
| 12 | 5 | 5 | 100 |
| 14 | 4 | 3 | 75 |
| 15 | 4 | 4 | 100 |
| 16 | 3 | 3 | 100 |
| 17 | 5 | 2 | 40 |
| 18 | 4 | 0 | 0 |
| 19 | 2 | 0 | 0 |

uteri. Vaginal smears were made only on the day of autopsy to establish whether or not heat existed at that time.

The results obtained (table 1) show that endometrial sensitivity is not present until the fourth day; no deciduomata were obtained in animals stimulated on the first, second, or third postpartum days. From the fourth to the sixteenth days sensitivity is present, deciduomata being obtained in 32 of 36 experiments. The distribution of the four negative results, two on the fourth, one on the tenth, and one on the twelfth days, is such that it seems very unlikely that any difference in sensitivity exists during the period from the fourth to sixteenth days inclusive. The sensitivity rapidly diminishes after this; deciduomata were found in only two out of five cases stimulated on the seventeenth day. None were present in four animals stimulated on the eighteenth day, and none in two

stimulated on the nineteenth day. It is also of interest that in most cases the deciduomata produced were smaller than those previously obtained in the sterile horn of unilateral pregnancy (Lyon and Allen, 1936).

The results obtained are of considerable theoretical interest for several reasons. They show that during lactation the corpora lutea may remain functional for at least sixteen days, since the endometrium remains sensitive throughout this period (excepting the first three days required for the development of the sensitivity). It is well established that during pregnancy the corpora are functional to almost the end of gestation yet the period of endometrial sensitization is limited to the fourth, fifth, sixth, and seventh days at most. What is the explanation of this difference? It seems probable that during pregnancy the estrogen level may be considerably higher than during lactation. During pregnancy extensive mucification of the vagina occurs, a change which can be produced in castrated animals by injection of sub-cornifying amounts of estrone (Meyer and Allen, 1933) or better by giving a suitable combination of estrogenic and progestational (corpus luteum) hormones (Allen and Meyer, 1935; Korenchevsky and Hill, 1937; Klein, 1937). Also during the latter half of pregnancy certain changes take place in the endometrium (Allen, 1931) which are characteristic of pregnancy, and which Korenchevsky and Hill (1937) have shown can be produced by giving a combination of estrone and progesterone. There seems little doubt that during the latter part of pregnancy both hormones are acting under normal conditions. During lactation extensive mucification of the vagina does not occur, and in addition the endometrium remains much as it is six to seven days after mating. It seems probable, therefore, that during lactation the estrogen level remains low, and that this, combined with persistency of the corpora lutea, maintains the endometrium in a sensitive state.

## CONCLUSIONS

During lactation in the rat the endometrium remains sensitized from the fourth to sixteenth days after delivery. Sensitivity rapidly diminishes on the seventeenth day and is not present on the eighteenth and nineteenth days.

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# THE RÔLE OF EPINEPHRINE IN THE LACK OF RESPONSE TO INSULIN IN DIPHTHERIA INTOXICATION

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Received for publication February 10, 1938

It is well known that in certain infections and in poisoning with diphtheria toxin the action of insulin on the blood sugar is reduced or absent. This phenomenon has been ascribed by various authors to antagonistic actions of the adrenal, the thyroid and the pituitary glands. Corkhill (1932) found that in young fasting rabbits in the early stages of poisoning with diphtheria toxin there was an early recovery from hypoglycemia which he termed "resistance to insulin." This was abolished by ergotoxin which inhibits the action of epinephrine. Recently Zucker and Berg (1937) have shown that recovery from insulin hypoglycemia in normal animals is hastened by a secretion of epinephrine and Corkhill's findings are therefore probably to be regarded as due to an intensification of the normal process. This intensification may mean one of two things: Either a smaller stimulus is sufficient to call forth the secretion of epinephrine, or the epinephrine produced, although no greater in amount than in the normal animal, is more effective. In favor of this latter possibility, i.e., that carbohydrate metabolism is somehow sensitized to epinephrine by the toxin, Lawrence and Buckley (1927) report that glycogenolytic agents such as adrenalin and posterior pituitary extract cause an increased response. These authors, however, conclude that the essential factor is overaction of the thyroid and adrenal, and base their conclusion on two arguments. First, they find that these animals show a prelethal rise of blood sugar and that this is prevented by ergotamin. Secondly, on histological examination of the tissues they find, in agreement with Cramer (1926), that the toxin produces exhaustion and marked degeneration of the thyroid and adrenal, "conditions which are usually preceded at some stage by stimulation and over-activity." Apart from the hazardous nature of deductions of function from the histological picture, these pathological findings might quite well be interpreted to mean that in the earlier stages the "insulin-resistance" in Corkhill's sense was due to overaction of these glands, but that in the later stages the exhausted glands were no longer able to respond to stimulation by a secretion of their hor-

mones and that some other cause must be sought. Moreover degeneration of other organs involved in carbohydrate metabolism, such as the liver, muscles and pituitary (Gagyi, 1934) has been reported.

This work was therefore undertaken to throw light on the following problems: Is the absence of response to insulin in the later stages of poisoning due to the same cause as the more rapid recovery from hypoglycemia in the earlier stages and, if epinephrine is involved, does the toxin sensitize carbohydrate metabolism so that the blood sugar response to epinephrine is increased, or does it act by sensitizing the adrenal gland?

METHODS. Healthy rabbits were used, weighing as a rule between 2 and 3 kgm. The general plan of the experiments was as follows: A control curve was made by giving insulin subcutaneously in doses between 0.37 and 0.51 unit per kgm.; blood was obtained either from the ear vein or by cardiac puncture at suitable intervals and analyzed for sugar by the method of Hagedorn and Jensen. Following this 1.25 to 1.5 M.L.D. of diphtheria toxin[1] per kgm. was given subcutaneously. This dosage was sufficient to kill about two-fifths of the animals by the fourth day. On the morning of the 3rd day (i.e., about 44 hours after giving the toxin) insulin was given in the same dosage and the blood sugar curve followed, some of the animals being given ergotoxin in order to prevent the action of epinephrine. In another series epinephrine was substituted for insulin, but in this series ergotoxin was not used.

RESULTS. Figure 1A shows the control curves: insulin in the healthy animal. Figure 1B shows similar curves obtained in the animals which had been given toxin; instead of a fall of blood sugar we now usually obtain a rise. Figure 1C is similar to figure 1B except that these animals had been given 0.65 mgm. of ergotamin per kilogram subcutaneously half an hour before giving insulin in order to abolish the effect of epinephrine. (In the case of the points surrounded by a circle blood was obtained by cardiac puncture. Insulin was given intravenously in two experiments shown by broken lines; this, of course, produces a more rapid fall in the blood sugar.) A comparison with figure 1A shows that after ergotoxin with two exceptions insulin is approximately as effective as in the normal animal. From this it is to be concluded that the lack of response of the blood sugar to insulin in the poisoned animals is due in most cases, though possibly not in all, to an antagonistic effect of epinephrin. The exceptions all belong to the group in which insulin was given subcutaneously and circulatory difficulties may have prevented its absorption; it was noted that it was unusually difficult to obtain 0.2 cc. of blood from the ear vein in these cases. Moreover the action of ergotoxin is somewhat uncertain and a few negative results are not of great significance.

[1] We are indebted to Mr. J. Stockard of the State Laboratory of Hygiene, Raleigh, N. C. for a supply of diphtheria toxin.

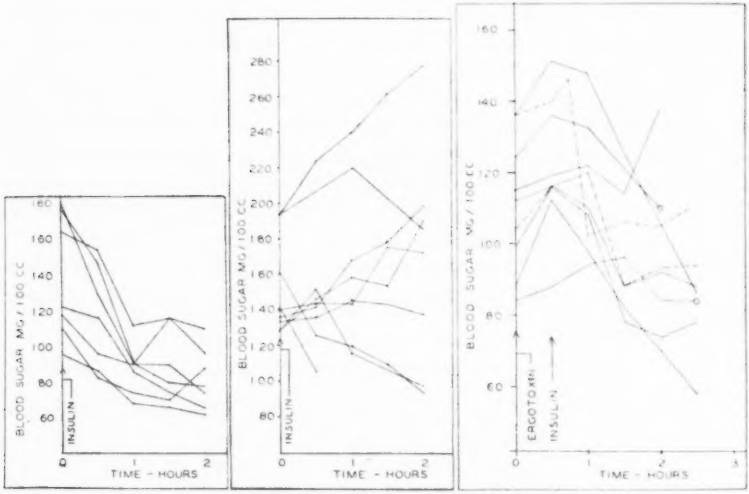


FIG. 1A FIG. 1B FIG. 1C

Fig. 1

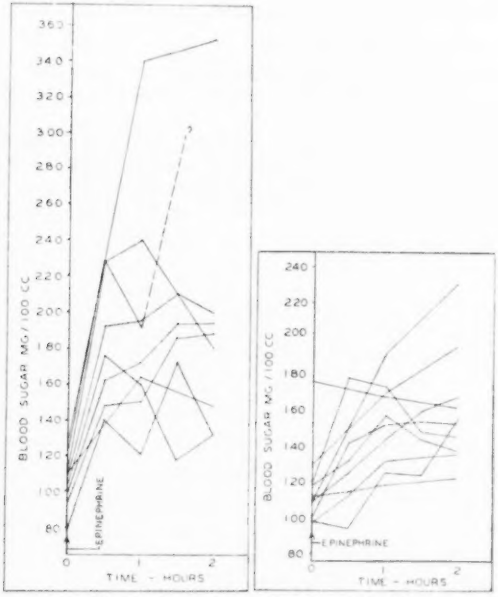


FIG. 2A FIG. 2B

Fig. 2

To determine whether such animals are unduly sensitive to epinephrine a second series was made. Epinephrine was given intravenously in dosages of 0.1 cc. of 1:1000 solution. Figure 2A gives the curves obtained from normal animals and figure 2B those from the same animals after receiving the toxin. It is evident that on the whole there is a somewhat diminished response in the poisoned animals; this is probably to be attributed to the decrease in liver glycogen which is found in this condition (Yannet and Darrow, 1933). From this it is concluded that, since the ergotoxin experiments show that the main antagonistic action is due to epinephrine and since these animals show, if anything, a diminished response to it, there must be a marked lowering of the threshold for stimuli to the adrenal medulla.

## SUMMARY

Insulin fails to lower the blood sugar in most animals poisoned with diphtheria toxin; this power is restored by ergotoxin. In the poisoned animals epinephrine produces a somewhat diminished effect on the blood sugar and it is concluded that the toxin markedly lowers the threshold for stimuli which induce the secretion of epinephrine.

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# THE OCCURRENCE OF ACETYLCHOLINE IN GASTRIC JUICE[1]

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Received for publication January 20, 1938

The literature definitely indicates that acetylcholine (a.c.) serves as the mediator of nerve impulses through the vagi to the gastro-intestinal tract (McSwiney and Robson, 1931; Feldberg and Rosenfeld, 1933; Chang and Gaddum, 1933; Kahlson, 1934; Feldberg and Kwiatkowski, 1934; Dale and Feldberg, 1934; and Bunting, Meek, and Maaske, 1935). If a.c. is produced in the stomach wall, it is reasonable to postulate that it might be found in the gastric juice. Accordingly experiments have been performed to ascertain whether a.c. is present in the gastric secretion.

Our studies on stomach juice have revealed several interesting points; we have found stomach juice to contain (1) a.c.; (2) another substance which lowers the blood pressure even after administration of atropine and contracts the isolated strip of intestine; and (3) a substance with apparently toxic properties relaxing the intestinal strip.

*Preliminary experiments.* Obviously the question whether or not gastric juice destroys a.c. had to be answered first. To do so, known quantities of a.c. solution were mixed and incubated with gastric juice of man and dog. The mixture was then neutralized, diluted and tested on the eserinized rectus abdominis muscle of the frog. No destruction of a.c. occurred. The same result was obtained when samples of juice were buffered (phosphate buffers) at pH ranging from 5.2–8.5. Using phosphate and glycine buffers, it was also found that saliva did not contain a.c. esterase. Thus if a.c. is present in the gastric juice it should be possible to detect it.

*Method of extraction.* It was found necessary to concentrate the gastric juice, which procedure removed much protein and salt. The following method of extraction was finally adopted: 250 cc. of fresh gastric juice was filtered by suction, adjusted to about pH 4 and then concentrated at 40°C. under $CO_2$ and reduced pressure to about 10 cc. The concentrate was poured into 25 volumes (about 250 cc.) of acetone and filtered after standing for $1\frac{1}{2}$ hours. The precipitate was redissolved in a few

[1] Aided by the Oscar Aberle and Otto Baer Funds.

[2] Fellow of the Emergency Committee in Aid of Displaced Foreign Physicians.

cubic centimeters distilled water, and the precipitation repeated. The combined acetone filtrates were dried on a sand bath at 70°C. The residue was taken up with a few cubic centimeters of distilled water and the pH readjusted to 4; then it was filtered and made up to 5 cc. The extracts thus obtained were fifty times the concentration of the original gastric juice. In order to control our chemical procedure, a known amount of a.c. in Ringer's solution was similarly treated and recovered.

Three methods of assay were used: I, the leech; II, the blood pressure; and III, the guinea pig's ileum.

*I. Results with the leech method.* As a specific test for a.c., the leech method was used (Minz, 1932). A strip of leech (hirudo medicinalis) was suspended in 6.5 cc. of Ringer's solution[3] to which 0.5 cc. of eserine salicylate 1:50,000 was added; the concentration of eserine in the bath amounted to 1:700,000. The mixing of the test solution with the Ringer bath took place by bubbling through $O_2$. The addition of 2 cc. of a Ringer solution, acidified to pH 4, to the bath did not affect the leech muscle.

We obtained contraction of the leech with practically every extract of canine or human gastric juice. Figure 1 shows that this contraction occurred only after eserinization. This can be considered as evidence for the presence of a.c., which evidence was further confirmed by the esterase test; the contracting substance was destroyed completely after incubation with 0.2 cc. of fresh dog's blood.

The quantitative estimation of a.c. was made difficult by the toxic effects of the extracts. Larger and repeated doses rendered the leech entirely non-responsive to a.c. and recovery did not occur after repeated washings. Therefore, we had to change the method of "bracketing" used by Dale and Feldberg in the following way: First we recorded several contractions to known amounts of a.c. within the limits of which we expected to find the a.c. concentration of the extracts, and then the extract was tested. The concentration of a.c. varied from 0.024 to 0.33 gamma per 100 cc. of gastric juice; this concentration amounts to approximately one-tenth of that found by Dale and Feldberg (1934) in the perfusate of the stomach without vagal stimulation.

*II. Results of blood pressure assays.* The following experiments were done in order to control the a.c. assays on the leech. The usual method for blood pressure assay was employed on dogs, cats and rabbits under ether anesthesia. The effects of the extracts were compared with those of known solutions of a.c. before and after atropine.

The extracts caused a drop of blood pressure which was only partly abolished by atropine. This indicates that our extracts contain a.c. which is inhibited by atropine and a second depressor substance which is not inhibited by atropine. In rabbits the second substance caused

[3] NaCl 0.7, KCl 0.04, $CaCl_2$ 0.02, $NaHCO_3$ 0.02 or 0.025 per cent

a drop of blood pressure which was entirely different in character from the one produced by a.c. This is demonstrated in figure 2; injection of 1 cc. of the extract produced an immediate fall of blood pressure similar in character and depth to the one following 1.2 gamma a.c.; before the blood pressure had quite returned to its control level, the second atypical drop occurred. It was much less acute and of longer duration than the primary drop. Following the injection of 4 mgm. of atropine both 1.2 gamma a.c. and 1.0 cc. of the extract caused a small rise of blood pressure; in case of the extract this was followed however by a drop quite identical with the secondary atypical one observed before atropine. Thus, figure 2 demonstrates the presence of a second depressor substance which is wholly unaffected by atropine and differs from a.c. in the shape of the drop of blood pressure caused by it. In atropinised cats and dogs the second depressor substance caused an evanescent drop of the same shape as that following injection of a.c.; thus a simple additive effect had occurred before atropine. The diminution of the drop of blood pressure caused by atropine was measured and corresponded roughly to the a.c. content of the extract. The values thus obtained checked with those calculated from our tests on the leech which is sensitive to a.c. only.

The above experiments on blood pressure qualitatively and quantitatively confirm our results obtained on the leech as to the constant occurrence and the concentration of a.c. in gastric juice. They also demonstrate the presence of a second vaso-depressor substance. Since histamine does not lower blood pressure in rabbits (see fig. 2), the atropine-resistant depressor effect of the extract cannot be due to histamine; neither can it be ascribed to kallicrein, which is insoluble in acetone (Kraut, Frey and Werle, 1930). The possibility remains that the second vaso-depressor substance in the gastric juice is identical with or closely related to substance P of Euler and Gaddum (1931), especially since Euler and Gaddum had found that the stomach contained large amounts of P. This possibility was checked in experiments on the intestinal strip which P is known to contract (v.i.).

*III. Results on the strip of guinea pig's ileum.* The usual method was employed: the composition of the Ringer solution was: NaCl 0.9, KCl 0.02, $CaCl_2$ 0.02, $MgCl_2$ 0.01, $NaHCO_3$ 0.04, glucose 0.1 per cent. Both a.c. and P are known to cause contraction of the isolated strip. However, it was soon found that the extracts contained a contracting and a relaxing factor. The relaxing substance obscured the results not only by relaxation but also by changing the type of response of the ileum to drugs.

*A. Contraction effect.* The presence in our extracts of two different contracting substances was shown by two types of contraction of the ileum. Either a sustained rise of tone occurred as shown in figure 3 which did not differ at all from an a.c. contraction; or, a slow rise in tone was seen with

contractions setting in rather late and gradually increasing in number and size (fig. 4). Curves similar to ours in figure 4 have been published by Euler and Gaddum (1931) and Euler (1936) for substance P. In order to differentiate between the contracting effect of a.c. and substance P. the esterase test was employed. P is known to be unaffected by blood enzymes. When fresh dog's blood had been added to the extracts, a definite decrease of the contraction effect resulted. The remaining contracting agent was assumed to be P. Finally, the responses of both the leech and the ileum to the same extract H and a.c. were compared as illustrated in figure 5. The effect of 1 cc. of H in the leech test corresponded to that of approximately 0.02 gamma a.c. whereas in the gut test 0.2 cc. H caused a much bigger contraction than 0.02 gamma a.c. This quantitative difference is due to the additive effects of a.c. and P in the case of the gut, and to the specificity of the leech test for a.c.

Using chemical methods no evidence was found that two different contracting substances were present in the extracts. The contracting fraction dissolved in trichloracetic acid and remained in the aqueous

Fig. 1. Dorsal muscle of leech. Acetylcholine effect of extract before and following eserinisation.

A: extract of dog's gastric juice (gastrostomy) after histamine-acetylcholine subcutaneously. Concentration 1:61.

Fig. 2. Rabbit's blood pressure. Effects of acetylcholine and extract before and after atropine.

ZL: extract of dog's gastric juice (gastrostomy) after histamine-acetylcholine subcutaneously. Concentration 1:45.

Fig. 3. Guinea pig's ileum. Destruction of contracting factor by boiling with NaOH.

E: extract of dog's gastric juice (Heidenhain pouch) after histamine-pilocarpine subcutaneously. Concentration 1:4.

Eb: the same extract after boiling with N NaOH.

Fig. 4. Guinea pig's ileum. Two different types of contraction following two different extracts.

O: extract of dog's gastric juice (Heidenhain pouch) after histamine pilocarpine subcutaneously. Concentration 1:6.

V: extract of dog's gastric juice (Heidenhain pouch) after histamine pilocarpine subcutaneously. Concentration 1:8.

Fig. 5. Comparison of the contracting substances on the leech and on guinea pig's ileum.

H: extract of dog's gastric juice (gastrostomy) after histamine pilocarpine subcutaneously. Concentration 1:50.

Fig. 6. Guinea pig's ileum. Effect of R.L. on acetylcholine contraction.

Tri: extract of human gastric juice after Ewald meal. Concentration 1:12.

Fig. 7. Guinea pig's ileum. Inhibition of acetylcholine. Effect by R.S. but not by adrenaline.

R2: extract of human gastric juice after Ewald meal. Concentration 1:15. Adrenaline crystalline.

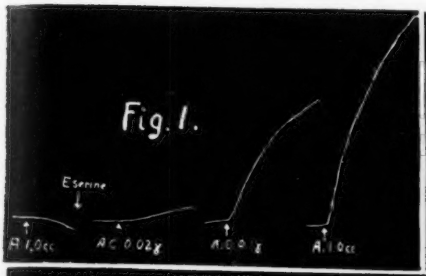
Fig. 1.
Eserine
A 1.0cc
A.C 0.02γ
A.C 0.0 γ
A 1.0cc

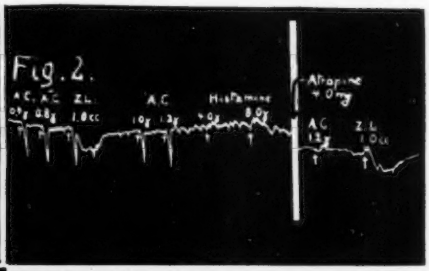
Fig. 2.
A.C. A.C. Z.L.
0.05γ 0.08γ 1.0cc
A.C
1.0γ 1.2γ
Histamine
0.02γ
Atropine
+0 mg
A.C
1.5γ
Z.L
1.0cc

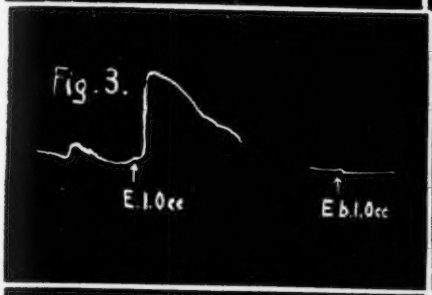
Fig. 3.
E 1.0cc
E b 1.0cc

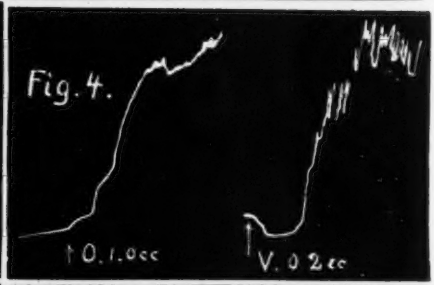
Fig. 4.
0.1.0cc
V. 0.2cc

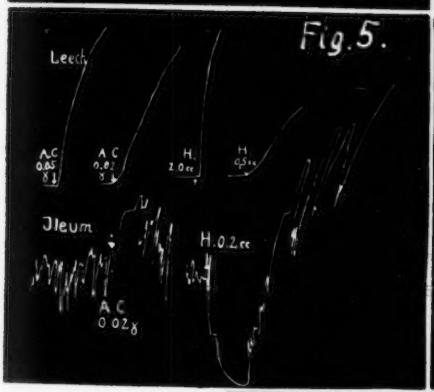
Fig. 5.
Leech
A.C
0.05
γ
A.C
0.05
γ
H.
2.0cc
H
0.2cc
Jleum
H. 0.2cc
A.C
0.02γ

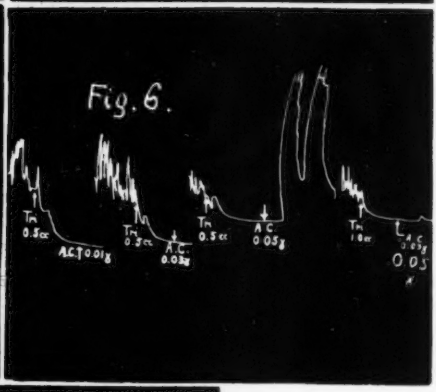
Fig. 6.
Tn
0.5cc
ACT 0.01γ
Tn
0.5cc
A.C.
0.02γ
Tn
0.5cc
A.C.
0.05γ
Tn
1.0cc
A.C
0.05γ

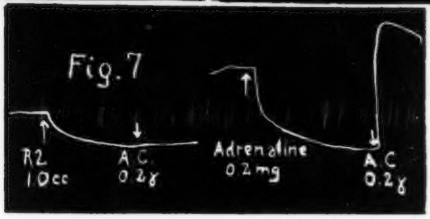
Fig. 7
R2
1.0cc
A.C.
0.2γ
Adrenaline
0.2mg
A.C.
0.2γ

Figs. 1–7

portion after shaking with ether. It was completely destroyed by boiling with alkali as demonstrated in figure 3. The contracting factor was unaffected by boiling with N HCl but destroyed by boiling with concentrated acid.

The effects ascribed to substance P cannot be due to histamine, because this substance would not produce such curves as seen in figures 4 and 5, and would not be completely destroyed by boiling with N NaOH (fig. 3).

*B. Relaxing effect.* The relaxing factor in the extracts will be referred to as R.S. In some extracts R.S. only seemed to be present, in others the contracting effect could always be eliminated by boiling with alkali.

R.S. is soluble in alcohol, trichloracetic acid, and in acetone. Shaking the trichloracetic acid fraction with ether leaves R.S. in the aqueous portion. R.S. is wholly unaffected by boiling with alkali and is not precipitated by ammonium sulphate. Boiling with acid did not diminish the relaxing effect in most experiments.

Relaxation of the strip begins immediately after the addition of R.S., tone and contractions being affected equally. As with most relaxing substances the strip maintains its tone at a constant level after relaxation has occurred. If the extract contains the contracting factor besides R.S., the relaxing effect precedes the contraction as shown in the second curve of figure 4 and the last one in figure 5. During relaxation the gut is relatively insensitive to histamine and a.c. This inhibition follows quantitative rules. The tracings in figure 6 illustrate that 0.5 cc. of extract Tri supressed the stimulating effect of 0.01 and 0.033 but not of 0.05 gamma of a.c., while 1 cc. of the same extract inhibited the effect of 0.05 gamma of a.c. completely. The response of the strip to higher concentrations of histamine or a.c. in the presence of R.S. is altered in as much as rather violent contractions occur instead of the typical rise of tone; sometimes latent periods have been found which were not observed otherwise.

The possibility that R.S. may be related to one of the known substances which relax the isolated strip of intestine has been considered. Adenosine can be ruled out because it is destroyed by boiling with acid (Barsoum and Gaddum, 1935) and is almost insoluble in a higher concentration of acetone (Gaddum and Schild, 1934). The antagonism of adrenalin to a.c. and histamine has been thoroughly investigated by F. Bernheim (1931–1933). Adrenalin never shows the irregular contractions described above when given with these drugs; also the quantitative effects of R.S. and adrenalin are different as illustrated in figure 7. Furthermore, adrenalin as well as pressor substances with relaxing effect on the gut (described by J. B. Collip, 1928) are ruled out by the absence of pressor effects. R.S. has toxic properties because after adding it in too large amounts or too frequently the gut dies. This corresponds to the toxic effect observed

on the leech. We are not able to define the nature of R.S.; probably it is a product without physiological significance.

Discussion. The specific proof for the occurrence of a.c. in gastric juice is furnished by the following results of our experiments: the contraction of the eserinized leech muscle, the abolition of the drop of blood pressure by atropine, the quantitative agreement between the a.c. equivalents obtained by these two methods and finally, the complete destruction of the contraction effect on the leech and the partial destruction of the contraction effect on the intestinal strip by blood esterase.

It is improbable that the a.c. in the gastric juice is derived from the myenteric plexus or from the musculature of the stomach because most probably it would be destroyed by tissue or blood esterase before reaching the lumen of the stomach. One may therefore assume that it is produced in the gland cells and leaves these either by diffusion or with the external secretions. Since Dale and Feldberg have demonstrated an increase in a.c. in the venous blood following stimulation of the vagi the question remains whether the occurrence of a.c. in stomach juice is due to cholinergic stimulation of the gland cells, a point which bears further physiological and clinical investigation.

The occurrence of P or a related substance in gastric juice is proven by these facts: following atropine part of the depressor effect on blood pressure remains; in the rabbit two different types of vaso-depressor effects occur; the analysis of the contraction effect on the intestinal strip reveals two characteristic factors (a.c. and P). It follows from both the experiments on blood pressure and on the gut that the second factor (P) cannot be histamine. This is not in accord with the findings of Brown and Smith (1935) who reported a substance resembling histamine in the gastric juice using Best and McHenry's method of extraction. They excluded effects of a.c. by atropinisation and P certainly was destroyed by their method of acid extraction and boiling. On the other hand, histamine is soluble in acetone and therefore any histamine present in gastric juice should have entered our extracts. Since Brown and Smith generally worked on large amounts of gastric juice, a quantitative factor may possibly explain the difference. The substance in question satisfied all requirements of Euler's substance P and is probably identical with it. Euler attributes physiological significance to P as an effective factor for intestinal motility and the question arises whether the presence of this substance in gastric juice might serve some physiological function.

## CONCLUSIONS

1. Human and canine gastric juice does not destroy acetylcholine. Neither gastric juice nor saliva contains acetylcholine esterase.

2. Human and canine gastric juice constantly contains small amounts of acetylcholine.

3. Human and canine gastric juice also contains a second substance which lowers the blood pressure after atropine and contracts the isolated strip of intestine. It is either identical with or closely related to Euler and Gaddum's substance P.

4. Human and canine gastric juice contains a toxic substance which relaxes the isolated strip of intestine and renders it irresponsive to acetylcholine and histamine. This substance also depresses or abolishes the response of the leech to acetylcholine.

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# THE CONTRIBUTION OF THE AURICLES TO VENTRICULAR FILLING IN COMPLETE HEART BLOCK[1]

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Received for publication February 11, 1938

The importance of the auricular contribution to ventricular filling has not been made entirely clear, although it has long been the subject of investigation. Henderson (1, 2) ascribed only minor importance to the auricles in filling the ventricles, while Straub (3) later found as much as 60 per cent of ventricular filling contributed by the auricles. Wiggers and Katz (4) gave figures of from 18 per cent to 60 per cent under various conditions, but later Wiggers in his monograph (5) laid less stress on its importance.

These differences in results are due partly to the difficulty in properly recording and interpreting ventricular volume curves, as has already been pointed out (4), and partly to differences in experimental circulatory conditions and consequent variations in the factors which control auricular contribution. It was the purpose of this study to investigate some of these factors by correlating the relative contribution of auricular beats occurring at different times in the heart cycle with their $A_s$–$V_s$ intervals.

METHOD. Dogs averaging about 15 kilos were anesthetized with sodium barbital and the chest was opened widely using artificial insufflation of the lungs. In order to study the effect of a varying $A_s$–$V_s$ interval on the auricular contribution, complete A–V block was produced by crushing the common A–V bundle with a special clamp as described by Lewis (6), one blade of the clamp being inserted through the right subclavian vein into the right ventricle, and the other blade through the left subclavian artery into the left ventricle. The jaws of the clamp were then approximated on the intraventricular septum in the region of the common bundle, and sufficient pressure applied to produce complete A–V block. It was found necessary to leave the clamp in place to insure continuance of the block. The presence of the clamp caused no significant decrease in the vigor of the heart, and did not interfere with the subsequent taking of records. Simultaneous ventricular volume and aortic pressure curves were recorded photographically according to the technique used by Wiggers and Katz (4). The cannula of the Wiggers manometer was

[1] Aided by the Emil and Fanny Wedeles Fund for Cardiac Research.

inserted into the ascending aorta through one carotid artery near its origin from the innominate artery. Respiration was temporarily stopped while records were being taken.

*Interpretation of curves.* Figure 1 is a portion of a record taken during complete A–V block; it illustrates the measurements made on the records to determine what we consider the true auricular contribution in each

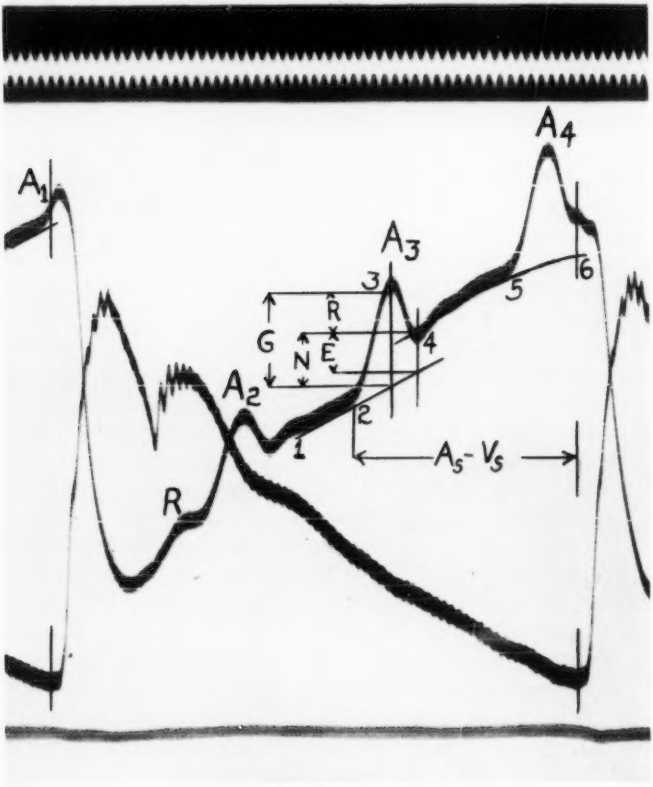


Fig. 1

case. Four types of auricular beats are shown ($A_1$, $A_2$, $A_3$, $A_4$). The beat marked $A_3$ which begins at about the middle of diastole is the type most frequently encountered in these experiments, so it will be analyzed in detail first. Here, as in all other beats measured, the $A_s$–$V_s$ interval is taken from the beginning of the auricular wave to the beginning of ventricular systole as indicated by the beginning of isometric contraction (the preliminary vibration) in the aortic pressure curve. The portion

of the volume curve marked *1–2* represents steady filling of the ventricles due to the inflow of blood from the venae cavae and pulmonary veins. At *2* auricular systole begins, and *2–3* represents the accelerated ventricular inflow caused by the auricular contraction. During this auricular ejection phase, it may be assumed that the inflow from the veins continues at about the same rate as before, so that the total amount of blood injected by the auricles alone into the ventricles, which may be called the "gross auricular contribution," is that indicated by *G* in figure 1. Actually the ventricular inflow from the veins during the interval *2–3* is probably decreased or possibly stopped completely by the contraction of the auricles, so that the gross auricular contribution may be greater than shown on the diagram. During the interval *3–4*, the ventricular volume rapidly decreases, indicating a back flow from ventricles to auricles. The gross auricular contribution minus the amount of regurgitation gives the total amount of blood left in the ventricle by the auricular contraction; this "net auricular contribution" is indicated in the figure by *N*. This, however, is not the true auricular contribution: the true "effective auricular contribution" is the vertical distance *E* from the point *4* in the figure to the forward projection of the slope *1–2*. This we consider the most accurate measure of the useful filling accomplished by the auricles, since if there had been no auricular beat at this moment, the filling of the ventricles would have continued at the same rate as indicated by the projected slope *1–2*. This assumption seems justified since the slope *4–5* after the auricular beat is approximately the same as the slope before. In most of the beats measured, the slope of the curve before and after the auricular wave remained the same; in a few beats occurring early or late in diastole the slope did change, but in these instances it was possible by interpolation to determine with sufficient accuracy the bottom point from which to measure *E*. In a few beats the effective contribution could not be measured because of distortion of volume curve by artefacts (e.g., $A_2$ in fig. 1). It will be seen that at the point *4*, the curve swings somewhat below the line *4–5*. This dip follows every auricular beat and is merely a free overswing of the air system used to record the volume changes. Consequently the point *4* is taken at the intersection of the mean slope, *4–5*, and the vertical line through the end point of the auricular wave.

The point marked $A_1$ is the beginning of an auricular beat which starts just 0.01 second before ventricular systole begins. In this case the contribution of the auricles is practically nil, since ventricular systole begins before the auricles eject any appreciable amount of blood into the ventricles. Here the effective contribution is merely the vertical difference in height between the level of the volume curve at the beginning of isometric contraction and the level at this instant of the projected slope of the curve before the auricular beat.

The auricular wave $A_2$ begins very early in diastole where, as Wiggers and Katz have shown (4), the ventricular volume curve is distorted and unreliable. Here it is impossible to determine the gradient of the curve before auricular systole begins, and so the effective contribution of this auricular beat can be only roughly estimated.

The fourth auricular beat in figure 1 ($A_4$) begins 0.13 second before ventricular systole, so that the end of the auricular wave coincides almost exactly with the beginning of isometric contraction. Here of course one cannot determine the slope of the filling curve after the auricular beat, since filling is stopped by the onset of ventricular systole, but the true effective contribution of this auricular beat can be closely estimated by measuring vertically from point 6 to a point 2 mm. above the intersection of the volume curve with the vertical line through the end point of the auricular beat. The 2 mm. allowance is made for the depth of the natural

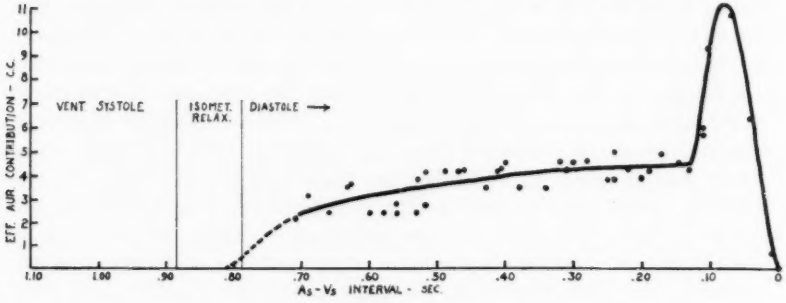


Fig. 2

overswing which is present here, as in $A_2$ and $A_3$, but obscured by the onset of systole.

The foregoing discussion of figure 1 serves to illustrate the various types of auricular beats encountered and the methods used in determining the effective contribution of each beat.

Results. The effective contributions of a series of auricular beats occurring at various times during the heart cycle in one complete experiment were measured and plotted against the corresponding $A_s$–$V_s$ intervals; the curve obtained is shown in figure 2. The point 0 at the right hand end of the abscissa scale represents the beginning of ventricular systole, the $A_s$–$V_s$ intervals being measured back from this point. The cycle lengths of all the beats used varied only from 1.07 to 1.12 seconds, with an average of 1.10 second; consequently the point A represents the beginning of the previous ventricular systole. $B$ is the beginning of diastole, and the interval $B$–$C$ is the period of isometric relaxation.

The earliest point on the curve which could be measured has an $A_s$–$V_s$

interval of 0.71 second. The dotted portion of the curve is merely an interpolation. During the interval from 0.71 to 0.13 second, the auricular contribution shows a tendency to increase from about 2.3 cc. to about 4.5 cc. The scatter of points is admittedly rather large, but the mean curve is sufficiently accurate to show the general trend. As the $A_s$–$V_s$ interval decreases from 0.13 second to 0, the auricular contribution rises sharply to about 11 cc. and then falls rapidly to zero.

In figure 3 the $A_s$–$V_s$ interval of each auricular beat is plotted against the gross auricular contribution (top curve), the amount of regurgitation into the auricles during auricular diastole (bottom curve), and the net auricular contribution (middle curve). The ordinates of this last curve represent the differences between the ordinates of the other two curves.

Discussion. The gradual increase of the effective contribution as the $A_s$–$V_s$ interval shortens is due in part at least to the fact that the gross auricular contribution increases as the interval shortens; that is, the

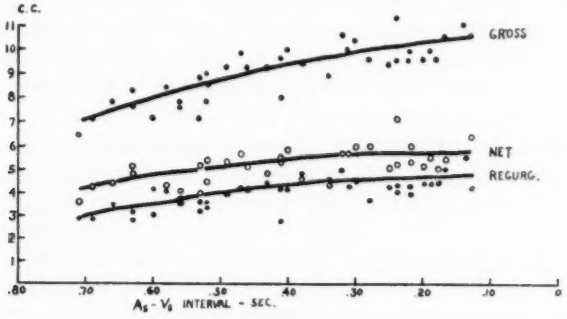


Fig. 3

auricular beats occurring late in diastole inject more blood into the ventricles than do the earlier beats. This is shown in the top curve of figure 3. The gross contribution is seen to increase from about 7.2 cc. to about 10.7 cc., an increase of 3.5 cc. It is difficult to offer an explanation for this increase because of the complexity of the factors involved; however, one explanation which suggests itself is based on the fact that the leaflets of the A–V valves are more closely approximated when the flow of blood past them is rapid. Thus, early in diastole when the $A_s$–$V_s$ interval is long, the ventricles are filling rapidly and the approximated valves offer a greater resistance to auricular ejection than they do later in diastole when ventricular filling is slower.

Over the same $A_s$–$V_s$ range, however, the effective auricular contribution increases from 2.3 cc. to 4.5 cc., an increase of only 2.2 cc. This is partially accounted for by the fact that as the gross auricular contribution increases, the amount of regurgitation into the auricles during auricular diastole

also increases, though at a slower rate (lower curve, fig. 3). Here the mean value for the amount of regurgitation is seen to rise from 2.8 cc. at an $A_s$–$V_s$ interval of 0.71 second to 4.8 cc. at an interval of 0.13 second, an increase of 2 cc. The net auricular contribution increases only 1.5 cc. (3.5 cc. minus 2.0 cc.) in this interval (middle curve, fig. 3).

As the $A_s$–$V_s$ interval shortens, the effective contribution approaches but remains less than the net contribution. As shown in figure 1, this is due to the fact that at the shorter $A_s$–$V_s$ intervals the general slope of the volume curve becomes less and so the difference between N and E becomes smaller. (Compare $A_2$ with $A_4$.) Thus the slowly rising portion of the curve in figure 2 is accounted for by two factors: 1, the increase in the volume ejected by the auricles coupled with a smaller increase in the amount which regurgitates, and 2, the decrease in slope of the ventricular volume curve between auricular beats.

It is evident from the curves that the amount of regurgitation from ventricles to auricles is always less than the gross auricular contribution. Our data provide no explanation for this fact. The proportion of the gross contribution which does flow back into the auricles is greater in the auricular beats which occur late in diastole (short $A_s$–$V_s$ interval) than in those occurring early (long $A_s$–$V_s$ interval).

When the $A_s$–$V_s$ interval is shorter than the duration of one auricular beat (0.13 sec. in this case) the effective auricular contribution is markedly affected by small changes in the $A_s$–$V_s$ interval. As the interval shortens from 0.13 second to 0.08 second the beginning of ventricular systole encroaches upon the period of regurgitation, thus increasing both the net and the effective auricular contributions. At an $A_s$–$V_s$ interval of 0.08 second, the regurgitation is prevented altogether, and the effective auricular contribution rises to a maximum of about 11 cc. As the interval becomes still shorter, the period of auricular ejection is cut short by the ensuing ventricular systole so that the effective contribution of the auricle rapidly decreases to zero.

In the normally beating heart auricular systole begins at some time during the period represented in our curve as lying between 0.13 second and 0.08 second before ventricular systole. Here the dynamic importance of the auricles in increasing the initial ventricular volume and pressure is much greater than would be the case if the $A_s$–$V_s$ interval were longer or shorter. The importance of an $A_s$–$V_s$ interval short enough to reduce the period of regurgitation and thus increase the effective auricular contribution was realized by Wiggers and Katz (4).

Gesell (7), using changes in arterial blood pressure as a criterion of effective auricular contribution, found a correlation similar to ours between $A_s$–$V_s$ interval and auricular contribution. The optimum $A_s$–$V_s$ interval found by him, however, was somewhat shorter than that found in our results.

It must be borne in mind that the data presented in this paper were obtained from a heart with complete A–V block, a slow ventricular rate, and three auricular beats in each heart cycle. These conditions, however, interfere in no way with the principle of the analysis of the factors affecting the useful effective contribution of the auricles. The factors would have the same relative influence in a heart with normal rhythm. The maximum effective auricular contribution found in this preparation represented 21 per cent of the total ventricular filling; this of course does not preclude the possibility of this figure being considerably higher under some conditions in a normal heart.

SUMMARY

From ventricular volume curves recorded simultaneously with aortic pressure pulses in a dog with complete A–V block, an analysis is made of the dynamics of each auricular beat. The effective auricular contribution to ventricular filling is defined and its magnitude correlated with the $A_s$–$V_s$ interval. The effective auricular contribution is found to increase slowly as the $A_s$–$V_s$ interval decreases. When this interval becomes equal to the duration of auricular systole, the auricular contribution begins to increase rapidly, reaching a maximum at an $A_s$–$V_s$ interval equal to about half the duration of auricular systole, and falling off sharply to zero as the interval further decreases. The factors affecting this correlation are discussed.

I wish to acknowledge my indebtedness to Dr. L. N. Katz, at whose suggestion this study was undertaken, for his guidance.

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# THE EFFECTS OF INANITION ON TEMPERATURE REGULATION[1]

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Received for publication February 10, 1938

In the course of some studies in temperature regulation it became necessary to determine what effect, if any, malnutrition has on the ability of a cat to regulate its body temperature. In the voluminous literature on malnutrition there was found little work which was applicable to the problem at hand, although many workers have reported a decrease in the B.M.R. as a result of inanition. Further, the question has frequently been raised as to whether death occurs as a result of exhaustion of reserves or because oxidative processes cannot proceed at a fast enough rate at low body temperatures (usually not given). (Jackson, 1925.)

Hehir (1922) made some interesting observations as to the course of body temperature during the siege of Kut. He stated that "in chronic starvation the temperature tends to fall very gradually." Some of the Indian troops were found to have temperatures of 94 or 95°F. while on duty and in fatal cases the temperature was often less than 90°F. just before death.

In a preliminary study 4 cats were selected, two (cats 1 and 2) were in good condition but the other two (cats 3 and 4) were somewhat emaciated. The responses of these cats to cold box tests similar to those described by Ranson and Teague (1936) were within normal limits, i.e., the rectal temperature did not fall to a point below the normal range after 3 hours' exposure to a temperature of about 40°F. Then for 1 week food was withheld although fresh drinking water was supplied regularly. At the end of this period cold box tests were again made and it was found that the emaciated cats now gave marked falls (cat 3, 4.3°F., cat 4, 5.7°F.) while the other 2 responded normally (table 1).

Following preliminary experiments, five healthy, well-nourished cats were chosen. Two of these were deprived of food for one week (drinking water was supplied regularly) after which they received 100 cc. of milk daily and no water. The remaining 3 were deprived of food for 10 days after which 100 cc. of milk were given daily. A diet consisting solely of 100 cc. of milk is very inadequate for an adult cat and their weight loss

[1] Aided by a grant from the Rockefeller Foundation.

was rapid. The cats were tested in the cold box on the day the experiments began and at weekly or semi-weekly intervals thereafter, for periods varying from 5½ to 11 weeks. At the conclusion of the period hot box tests similar to those described by Ranson and Teague (1936) were performed, and then the animals were again placed on a full diet. After 2 weeks of this, cold box tests were again made.

It was found that all the cats gave abnormal responses to cold box tests after 5½ to 11 weeks when the weight losses had reached 42.5 to 52.7 per cent of their original weight (table 1). In three cats the rectal temperatures (taken in the animal room where the temperature varied from 60–70°F.) gradually fell, confirming the findings of Hehir (1922) in humans. The panting levels, as measured in the hot box tests made at the conclusion of each experiment, were all within normal limits (table 1).

TABLE 1

| CAT NUMBER | INITIAL COLD BOX TEST | | | FINAL COLD BOX TEST | | | HOT BOX | | |
|---|---|---|---|---|---|---|---|---|---|
| | Temperature at start | Temperature at end | Change | Temperature at start | Temperature at end | Change | Weight loss | Week | Panting level |
| | | | | | | | *per cent* | | |
| 1 | 101.3 | 101.0 | −0.3 | 100.2 | 99.8 | −0.4 | | | |
| 2 | 100.6 | 100.8 | +0.2 | 100.6 | 101.6 | +1.0 | | | |
| 3 | 100.4 | 100.6 | +0.2 | 100.2 | 95.9 | −4.3 | | | |
| 4 | 100.9 | 100.0 | −0.9 | 101.7 | 96.0 | −5.7 | | | |
| 5 | 101.9 | 101.4 | −0.5 | 99.5 | 97.4 | −2.1 | 42.5 | 5½ | 103.8 |
| 6 | 101.4 | 100.8 | −0.6 | 98.3 | 96.9 | −1.4 | 42.7 | 5½ | 103.8 |
| 7 | 102.1 | 100.8 | −1.3 | 99.2 | 95.2 | −4.0 | 48.7 | 7 | 104.9 |
| 8 | 100.0 | 100.3 | +0.3 | 101.6 | 97.8 | −3.8 | 52.7 | 7 | |
| 9 | 101.7 | 101.8 | +0.1 | 97.7 | <91.0 | >−7.7 | 49.8 | 11 | 104.6 |

The results in a condensed form are shown in table 1. The initial cold box tests show that there is a considerable range in rectal temperatures of adult cats (temperature at start of test) and that the temperatures recorded at the end of the test are all within this normal range. In 4 out of 5 cases (not considering the preliminary experiments) the temperature at the beginning of the final test is distinctly under the normal limits and in every case the rectal temperature at the conclusion of the test had fallen to a very low level. Cat 9 was not in a state of collapse even though the temperature was less than 91°F. at the conclusion of the test but, on the contrary, was as lively as in previous tests.

In the last column are shown the temperatures at which panting occurred. Although above the average, these are well within the normal limits as determined in numerous tests in this laboratory. Cold box tests made two weeks after the animals had again been placed on a full diet resulted in normal responses.

In figure 1 are shown the results of the cold box tests in a graphic form for cat 5. Each vertical line represents one test. The rectal temperatures (taken in the animal room) at the beginning of the tests are connected by a solid line. The rectal temperatures at the end of each cold box test are connected by an interrupted line and the weight losses (in per cent of original weight) are connected by a dotted line. It will be seen that this cat was able to maintain normal body temperature in the cold box at the end of 3 weeks at a time when it had lost 32.5 per cent of its original weight.

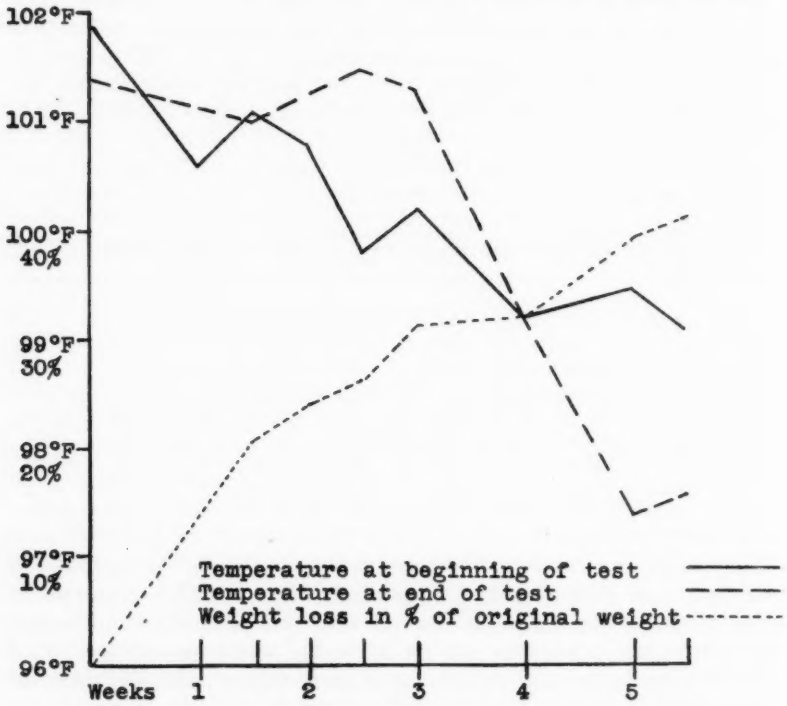


Fig. 1. Cold box tests on cat 5

But after the weight loss reached 33.7 per cent a slight drop occurred and when the weight loss reached about 40 per cent the response became abnormal, the temperature dropping to 97.4°F.

Two days after the final cold box test listed in the table, cat 8 accidentally received 100 grams of ground beef hearts in addition to the milk and two days later it gave a normal response to a cold box test. Two days after this, it was found in a moribund condition with a temperature of 82°F. and despite heating and fluids died. This cat (8) and cat 9 did

not show the gradual fall in rectal temperature that the others did. The eating of fecal material (which was not observed in the other cats) may have caused a toxic condition and a low grade fever.

## CONCLUSIONS

1. Normal well-nourished cats when placed on an inadequate diet after a preliminary period during which no food was given react normally to cold until the weight loss considerably exceeds 30 per cent.

2. The responses to heat are not interfered with by weight losses which cause abnormal responses to cold.

3. The rectal temperature of cats, which have lost considerably more than 30 per cent of their original weight as a result of an inadequate diet, drops to extremely low levels upon exposure to cold.

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# THE EFFECT OF PREVIOUS STIMULATION ON THE RESPONSIVENESS OF THE CAT'S NICTITATING MEMBRANE SENSITIZED BY DENERVATION

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Received for publication January 20, 1938

The increased sensitivity of denervated smooth muscle has been the subject of several recent investigations (cf. Cannon and Rosenblueth, 1937a). Its mechanism is nevertheless still obscure. Evidence from the difference in the effects of preganglionic and postganglionic denervation suggests that the increased sensitivity of denervated cells is conditioned directly by the release of these cells from the influence of nerve impulses (Simeone, 1937) and not by phenomena possibly associated with axon degeneration. Whether the nerve impulse conditions the effector cell exclusively by determining the presence or absence of sympathin within it, or whether it has other effects, as yet unknown, is not clear. It was thought of considerable interest, therefore, to test the responsiveness of the pre- or postganglionically denervated nictitating membrane (n.m.) to electric and chemical stimulation, after previously activating it by tetanization or by adrenalin injection, with the hope of throwing some light on the nature of the sensitization.

METHOD. Adult cats were used with the n.ms. denervated by excision of the superior cervical ganglion or by resection of a portion of the preganglionic cervical sympathetic trunk. The procedure was done on only one side when it was desirable to use the other as control. Isotonic records of the contractions of the n.ms. were made with writing levers exerting a tension of 4 grams and magnifying 20 times. Friction was reduced to a minimum by use of writing points modified slightly from those described by Liddell and Sherrington (1929). The membranes recorded either singly or simultaneously, one directly above the other. Dial anesthesia (Ciba, 0.75 to 0.80 cc. per kgm. intraperitoneally) was used, with one exception, in the acute experiments. The adrenal glands were removed in some cases at the time of the experiment, but not routinely. No related difference was observed in the results.

The experiments are divided into 3 groups. The first were performed to determine the effects of prolonged administration of adrenalin on the

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sensitivity of normal and denervated membranes to test doses of the hormone. The responses of the n.ms. to adrenalin were recorded in 8 animals before denervation, after denervation, and after long-continued administration of adrenalin. In 3, adrenalin was injected subcutaneously in divided doses (2 cc. of a standard 1:1000 solution, 3 times a day) for a period of 5 days. The sensitivity of the n.ms. was tested 6 hours after the last subcutaneous injection, when the denervated membrane was fully relaxed. In 5 other animals of this group the effect of continuous intravenous injections of adrenalin for periods of $\frac{1}{2}$ to $1\frac{1}{2}$ hours upon the responses of the n.ms. to test doses of adrenalin was studied. The injections were administered by means of the apparatus described by Colwell (1930). The syringe, filled by pressure from a Woulff bottle, was set to deliver 0.1 to 0.25 cc. of a 1:50,000 solution of adrenalin (Parke-Davis) every 15 seconds.

In a second group of 8 cats, the responsiveness of the normal and preganglionically denervated n.m. to test doses of adrenalin was studied before and after a prolonged tetanic stimulation applied to the postganglionic fibers by means of a Harvard coil with six volts in the primary circuit. The electrodes were applied to the postganglionic trunk after crushing the normal or decentralized ganglion. The vagus, hypoglossal, glossopharyngeal, and spinal accessory nerves were excised from about the site of stimulation. The vagus and sympathetic were cut again low in the neck. The responses of the nictitating membranes to adrenalin were recorded isotonically before and after tetanization lasting from 5 to 120 seconds. In three experiments isometric contractions were measured by means of an optical torsion-spring myograph. The results were the same as those from the isotonic records.

In the third group of experiments (8 animals) the effect of tetanus on the responses of the normal and sensitized n.m. to single or grouped maximal condenser discharges was determined. The stimuli were applied either through the same electrodes as the repetitive faradic current or directly to the membrane by means of a second pair of electrodes (cf. Simeone and Rosenblueth, 1934). Comparable results were obtained by the two methods. When the test shocks were applied directly to the membrane, small doses of curare were given to prevent indirect stimulation of neighboring striated muscles, and artificial respiration was used.

The test doses of adrenalin were always diluted to a standard volume of 1.0 cc. and injected into the femoral vein at a uniform rate requiring 10 seconds by the beat of a metronome.

RESULTS. A. *The responsiveness of normal and denervated nictitating membranes to adrenalin after prolonged administration of the hormone.* In all the animals studied (8) there was a slight but definite decrease in the magnitude of the contractions of the sensitized (denervated) n.m. to

test doses of adrenalin after its prolonged administration (fig. 1). A similar decrease was observed after the intravenous injection of adrenalin for shorter periods of time (fig. 8). On the other hand, the responsiveness of the normal n.m. was increased after administration of adrenalin for both ong and short periods (figs. 1 and 9). These results were quite consistent, and it may be concluded that the responsiveness of the sensitized denervated n.m. is diminished by the previous action of adrenalin, while that of the normal membrane is increased.

In some animals, test doses of adrenalin were given during the relaxing phase from a preceding large dose of the hormone. As a rule, the test

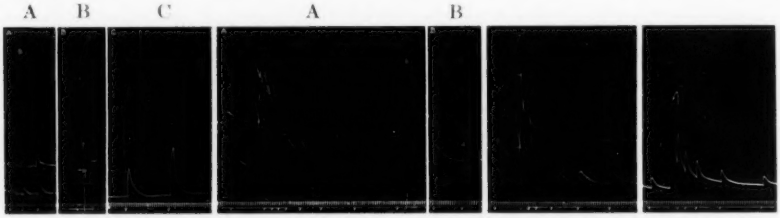


Fig. 1 Fig. 2 Fig. 3 Fig. 4

Fig. 1. Nembutal (0.7 cc. per kilogram intraperitoneally). Upper tracing, right, and lower tracing, left nictitating membrane (n.m.). *A*. Responses of normal membranes to 2.5, 5.0 and 7.5$\gamma$ adrenalin, injected intravenously. *B*. Responses to 2.5$\gamma$ adrenalin, same membranes as in *A*, but 2 weeks after excision of right superior cervical ganglion. *C*. Responses of same membranes to 2.5 and 5.0$\gamma$ adrenalin after subcutaneous injections of adrenalin in divided doses (cf. text) for 5 days. Time: 30 sec.

Fig. 2. In this and all figures to follow, anesthesia was dial (Ciba, 0.8 cc. per kilogram intraperitoneally). Upper tracing, contractions of normal n.m.; lower tracing, of denervated (sensitized) membrane. Second signal indicates injection of 100$\gamma$ adrenalin. All other signals represent injections of test doses of adrenalin (2$\gamma$) intravenously. Interval between *A* and *B*: 10 minutes. Time: 30 sec.

Fig. 3. Same experiment as in figure 2, but second signal here indicates a 90-second tetanic stimulation of postganglionic fibers. Time: 30 sec.

Fig. 4. Contractions of normal nictitating membrane in response to 90-second tetanic stimulation applied to postganglionic trunk (second signal) and to test doses of 2$\gamma$ adrenalin injected intravenously (all other signals). Time: 30 sec.

responses were potentiated on the denervated, sensitized side, but not on the normal side (fig. 2).

B. *The sensitivity to adrenalin of the normal and sensitized (denervated) nictitating membrane after prolonged tetanus.* Figure 3 is a record of the responses of the normal (upper tracing) and sensitized (by preganglionic denervation; lower tracing) n.ms. to test doses of adrenalin injected intravenously before and after tetanization of the postganglionic fibers for 90 seconds. It will be seen that the responses of both n.ms. are decreased immediately after the tetanus, but only for as long as the relaxation is still

incomplete. In figure 3 the responses returned to normal about 20 minutes after the end of the tetanus. In other experiments, in which fewer test doses of adrenalin were given, the relaxation was completed in less than 10 minutes and the responses returned to normal as relaxation ended, although the duration of the tetanizing stimulus and the size of the response to it were the same as in figure 3.

In several experiments tetanization on the normal side (fig. 4) was followed by an increase in the responses of the membrane to the test doses of adrenalin (cf. Wolff and Cattell, 1937), persisting until relaxation was complete.

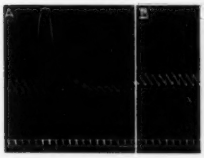


Fig. 5

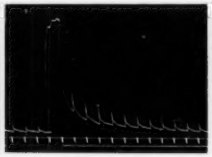

Fig. 6

Fig. 7

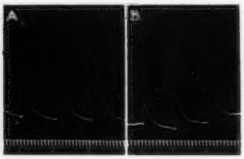


Fig. 8

Fig. 5. Dial, curare, artificial respiration. Responses of preganglionically denervated n.m. to single maximal condenser discharges directly to the membrane at rate of 1.0 per minute. At signal, response to tetanus applied to postganglionic trunk (ganglion crushed) for 120 sec. Interval between *A* and *B* represents rest period of 5 minutes. Time: 60 sec.

Fig. 6. Dial, curare, artificial respiration. Contractions of normal n.m. to direct application of maximal condenser discharges at rate of 2 per sec. every minute. At signal, response to tetanization of postganglionic fibers (ganglion crushed) for 60 sec. Time: 60 sec.

Fig. 7. Preparation similar to that in figure 5. Responses of preganglionically denervated membrane to single maximal condenser discharges applied to postganglionic trunk, begun at signal at rate of 1 every 30 sec. for 10 minutes. Time: 30 sec.

Fig. 8. Responses of sensitized n.m. (by excision of superior cervical ganglion) to 2.5, 5.0 and 7.0γ adrenalin, successively. *A*, before and *B*, after intravenous injection of adrenalin (0.15 cc. 1:50,000 adrenalin per 15 sec. for 1 hour). Time: 30 sec.

C. *The responses of the normal and sensitized nictitating membranes to nerve impulses after tetanus.* The responses of the preganglionically denervated (sensitized) n.m. to nerve stimulation are decreased after tetanus (fig. 5). The depression persists longer than the relaxation phase of the tetanic response, usually for longer than 30 minutes after a 90-second tetanus. The responses return to normal sooner if the test shocks are interrupted by a rest-period of 4 to 5 minutes. It is noteworthy that in these cases, in contrast to the responses to adrenalin, the decreased magnitude of contraction persisted even after relaxation of the membrane was complete.

The responses of the normal n.m. to nerve stimuli after tetanization of

the postganglionic fibers (6 animals) were invariably increased (fig. 6). This phenomenon has already been described by Cannon and Rosenblueth (1937b).

Discussion. The increased responsiveness of normal smooth muscle to adrenalin after previous activation by the hormone has already been reported (Tainter, 1929; Schlossberg, 1932; Bacq, 1936a). That the effect is not due to storage of the preceding doses of adrenalin and summation with the subsequent injections is probable since the potentiating effects on the test doses of adrenalin persist for at least several hours. Macht and Bryan (1935) report a decrease in the effectiveness of the oxidase of striped muscle in the presence of adrenalin. It is possible that, as Bacq (1936a) has suggested, adrenalin "in vivo" may have a similar depressant effect on oxidases of smooth muscle. Adrenalin and sympathin would then be more effective since their destruction would be delayed.

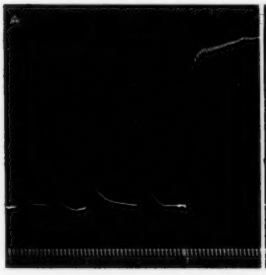


Fig. 9

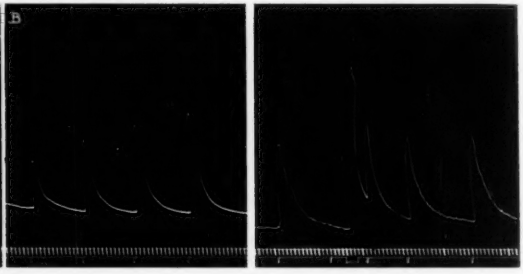


Fig. 10

Fig. 9. Responses of normal n.m. to test doses of adrenalin (2.5, 5.0, 7.5 and 10 successively). *A*, before and *B*, after constant intravenous injection of adrenalin as in figure 8. Prolonged injection started at fifth signal in *A*. Time: 30 sec.

Fig. 10. Preparation similar to that in figure 3. Contractons of sensitized membrane (by preganglionic denervation) in response to 4.0γ adrenalin before and after 90-second tetanus. Time: 30 sec.

The depressant effect of prolonged injections of adrenalin upon the responsiveness of denervated (sensitized) smooth muscle is less readily explained. Repeated injections of adrenalin were not well tolerated by the cats reported in the above experiments. For varying periods after the subcutaneous injection of adrenalin the animals were often prostrated, refused food, and lost weight. The effect, however, was not due to malnutrition or poor condition of the animals, because it occurred in cats after intravenous injections of the hormone for as short a period as 30 minutes. It is possible that adrenalin may decrease the permeability of the cell membranes of denervated smooth muscle cells and thereby decrease their responsiveness. It is more likely, however, that the prolonged injection of adrenalin has a depressant action directly on the contractile mechanism within the sensitized cells.

In contrast to the effects of prolonged administration of adrenalin, are the increased contractions elicited by test doses of the hormone when given during the relaxation from a preceding large dose (fig. 2). This was not noted in the normal, but only in the sensitized n.m. The same potentiation was observed in responses to nerve impulses, which was especially marked in the sensitized membrane, but seen also in the normal membrane. This is perhaps reasonably attributed to a persistence of adrenalin within the cells and to summation subsequently with further doses of adrenalin or with sympathin from nervous stimulation (cf. Cannon and Rosenblueth, 1937b). The effect lasts only as long as relaxation is incomplete. Presumably, the injected adrenalin is not yet destroyed during that period.

Tetanization of the n.m. sensitized by preganglionic denervation has no direct effect on the responsiveness of the membrane to test doses of adrenalin. The smaller responses after the tetanus are probably due to the incomplete relaxation of the organ (figs. 3 and 10). The responses are normal as soon as the membrane is fully relaxed. If no test contractions are elicited during the phase of relaxation, but only as soon as the membrane has returned to its original length, the responses to test doses of adrenalin may be normal as early as 10 minutes after a maximal 90-second tetanus. The depression, then, is not a function of the strength or length of stimulation, but is directly related to the degree of relaxation of the organ. This interpretation is at variance with that of Wolff and Cattell (1937), who attributed the decrease in magnitude of the responses immediately after tetanization to a depletion of some state or substance stored within denervated smooth muscle cells.

The responses of the normal n.m. to adrenalin are usually increased after tetanization, probably by the same mechanism increasing the responses to nervous stimulation after an injection of adrenalin. Why the phenomenon is not observed invariably is not known.

Cannon and Bacq (1931) first suggested that the increased sensitivity of smooth muscle after denervation may be due to a storage of sympathin or its precursor within the denervated cells. Wolff and Cattell (1937) suggest that hypersensitivity may result from an increase of some substance ("or state"), and Heinbecker (1937) from an increased storage of "epinephrine or of a substance on which it acts" within the cells. For any of these conditions to be possible, it is necessary to assume that the precursor of the mediator, $M$ (cf. Cannon and Rosenblueth, 1937a), is stored in an inactive form, while the receptor substance, $E$ or $I$, is in a state ready to combine with either $M$ or adrenalin. Some of the facts presented above support the storage theory. The decreased responses to nervous stimulation after tetanization and the return to normal some time after relaxation is completed, particularly after a rest period, can readily be explained on such a basis. The stored surplus might be depleted by the tetanus.

There is probably depletion of the receptor substance, since the responses to adrenalin are not decreased. The curious decrease in the responses to repeated single shocks applied to the preganglionically denervated membrane (fig. 7) can likewise be explained by depletion. The denervated adrenal gland, however, contains no more adrenine than the normally innervated gland (Elliott, 1912). It is possible that its content of some precursor of the hormone, as yet unidentified, may be increased.

The results presented above (cf. table 1) on the effects of adrenalin injections on the contractions resulting from test doses of the hormone are of interest in relation to other theories concerning the nature of sensitization by denervation. The evidence does not support the idea that the increased responsiveness of denervated cells depends upon their release from toxic effects of sympathin (Bacq, 1933). Indeed, prolonged injection of adrenalin renders normal smooth muscle hypersensitive. Increased permeability of the cell membranes has been stressed as a condition for increased sensitivity of denervated structures (Rosenblueth and Cannon,

TABLE 1

*Summary of the effects of previous activation on the responsiveness of the normal and the denervated nictitating membrane*

| CONDITIONING STIMULUS | RESPONSES TO ADRENALIN | | RESPONSES TO NERVE VOLLEYS | |
|---|---|---|---|---|
| | Normal membrane | Denervated membrane | Normal membrane | Denervated membrane |
| 1. Prolonged adrenalin injection | + | − | | |
| 2. Single adrenalin injection | 0 | + | + | ++ |
| 3. Prolonged tetanus | + | 0 | + | −− |

1936). This may be important as a contributing influence. The increased responses, however, to test doses of adrenalin injected during relaxation of the sensitized membrane after a large dose of the hormone, and the absence of this phenomenon in the normal membrane, cannot be explained readily on the basis of an increased permeability of the denervated cells. The effect of the injected adrenalin on the permeability of the effector cells should be at least qualitatively similar on the two sides, yet the responses to subsequent test doses of adrenalin are increased on the sensitized and not on the normal side. The potentiation on the denervated side might result from failure of these cells to destroy the hormone within or about them as readily as on the normal side. That a decreased capacity of denervated structures to destroy chemical mediator within them may play a rôle in the sensitization of denervated effectors has already been suggested (Rosenblueth and Cannon, 1936). Bacq (1936a) points out that if the responses of normal and denervated n.ms. are made equal,

then the durations of the relaxations are the same. Since smaller doses have to be used for the denervated side, however, it is difficult to see how this would be inconsistent with the theory that denervated smooth muscle is relatively incapable of destroying the mediator. Bacq (*loc. cit.*) suggests as an alternative theory that denervated smooth muscle cells are better able to "fix" phenolic amines.

Against the idea that sensitization by denervation is due to failure of the cells to destroy readily the chemical mediator is the fact that increased sensitivity after denervation is not specific, or, at best, the specificity is poor. Thus the sensitivity of denervated structures is increased even toward such inorganic ions as potassium (Bacq and Rosenblueth, 1934). That the phenomenon is not entirely non-specific, however, is indicated by the data reported by Bacq (1936b) that denervation does not sensitize to some aromatic, non-phenolic amines (e.g., ephedrine, phenylethylamine). Rosenblueth (1932) reported that denervation does not increase the responses of the n.m. to ergotoxine. Until more is known concerning the nature of the action of drugs on cells, it will be necessary to interpret with caution the non-specificity of increased responsiveness of denervated structures.

None of the theories suggested can alone explain the data presented above (table 1). The reactions of smooth muscle can be sensitized in a variety of ways, i.e., by denervation, by the antioxydants, including adrenalin itself, and by cocaine. The effects of these methods are grossly similar, but actually differ in important respects (cf. Bacq, 1936b and 1937). Sensitization, then, is not the result of a single uniform process, and it is possible that the effects of denervation itself are multiple. The permeability of denervated cells may be increased, their ability to destroy mediator may be impaired (cf. Brücke, 1937, who showed a decreased cholinesterase content in a denervated sympathetic ganglion), or there may be an increased store of the precursor of the mediator in the cells. Furthermore, the physical properties of the contractile system itself may be altered. The effects of denervation are somewhat analogous to the effects of physostigmine on the sympathetic ganglion; its principal effect is the protection of acetylcholine from destruction by cholinesterase, but it also has a non-specific effect in lowering the threshold for substances other than acetylcholine (Feldberg and Vartiainen, 1934), and even a synergistic action with acetylcholine has been suggested (Freud and Uyldert, 1935).

## SUMMARY

1. The prolonged administration of adrenalin increases the responsiveness of the normal nictitating membrane (n.m.) of the cat to test doses of adrenalin, but decreases the responsiveness of the n.m. previously sensitized by denervation (figs. 1, 8 and 9).

2. A single injection of adrenalin usually potentiates the responses to test doses of adrenalin in the sensitized n.m. (by denervation) but not in the normal membrane (fig. 2).

3. Tetanization of the preganglionically denervated n.m. does not decrease its responses to test doses of adrenalin. These responses are smaller than normal only while the membrane is still not completely relaxed. On the other hand, tetanization may increase the contractions of the normal membrane from test doses of the hormone (figs. 3, 4 and 10).

4. The responses to single nerve volleys are decreased immediately after tetanization of the preganglionically denervated n.m. The depression lasts longer than the time required for compete relaxation from the effect of the tetanus (fig. 5).

5. The bearing of these data on theories concerning the nature of sensitization of effectors by denervation is discussed (pp. 654–657).

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# THE EFFECT OF SYMPATHECTOMY ON GESTATION AND LACTATION IN THE CAT

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Received for publication January 20, 1938

Studies on animals after complete sympathectomy have shown that this division of the autonomic nervous system is quite dispensable for survival, at least when the animals are kept in a protective laboratory environment (Cannon, Newton, Bright, Menkin and Moore, 1929; Brouha, Cannon and Dill, 1936). There is evidence, however, that the reproductive function in some animals may be impaired after sympathectomy. Cannon and Bright (1931) observed deficiencies in lactation in the cat and dog after the sympathetic nervous system had been removed. Bacq (1932) made similar observations in the rat and, in addition, reported abnormality of parturition in 2 animals after partial sympathectomy. Abdominal sympathectomy in the male causes sterility. Erection is still possible after denervation of the internal genital organs of the male rodent, but ejaculation is absent (Bacq, 1931).

The importance of humoral influences in the physiology of reproduction is well known. Nelson (1936) has recently reviewed the data on the humoral control of the mammary glands. That nervous influences play an important rôle is indicated by the work of Selye (1934a), who suggested that afferent impulses from the nipples stimulate the mammary glands to activity by way of the pituitary. Lactation in the rat, then, may depend upon centripetal nervous and centrifugal humoral influences (Ingelbrecht, 1935). That efferent nervous effects may play a part in the control of the mammary gland, however, is suggested by the impairment observed in the cat, dog and rat, by Cannon and Bright, and by Bacq.

The deficiencies in lactation are belated (Cannon and Bright, 1931). Furthermore, they are not universal, but are seen only in some instances (Bacq, 1932). The factors responsible for the late appearance of the deficiency in lactation and for the sparing of the majority of animals are not known. It was therefore thought desirable to obtain more data on the effects of sympathectomy on the reproductive function.

METHOD. There are species differences in the mechanisms concerned with the control of the mammary glands (cf. Hesselberg and Loeb, 1937).

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The cat was chosen in these experiments because deficiencies in lactation have already been reported in this species (Cannon and Bright, 1931). For purposes of description, the animals are divided into four groups.

A. In 6 animals both lumbosacral chains were removed from the level of the diaphragm to the last pelvic ganglion inclusively. In 3 of these the right adrenal gland was removed and the left denervated.

B. In 3 animals, both stellate ganglia were excised.

C. The entire left paravertebral chain from the stellate ganglion to the last pelvic ganglion inclusive, and the right adrenal gland were removed in a third group of 4 animals.

D. In another group of 4 animals a complete sympathectomy was done according to the method described by Cannon *et al.* (1929). All operations were done aseptically under ether anesthesia.

Observations were made of the estrous behavior of these animals. During the 4 months following completion of the operations, several animals came into heat, and, of the entire group, 7 were successfully mated. Detailed records were kept of the animals' behavior and of the condition of the mammary glands during the pre- and postpartum periods, but the breasts were not biopsied at this time.

Ten to 12 months later (referred to below as the second period of observation) successful matings took place in 7 of the cats. Of these 7, 4 had been pregnant during the observation period of the preceding year. The behavior of the animals during and after pregnancy was studied. The condition of the breasts was noted at regular intervals, and whole breasts were removed in pairs for histological study at the 6th week of pregnancy, 1 week postpartum, and again 3 weeks later in all the animals except one. This one animal died at parturition and will be described fully below. The right and left breasts of the same segment were excised for comparison in all the animals except those in which the lumbosacral chains or the stellate ganglia alone had been removed. In these, one abdominal and one thoracic breast were removed from the same side.

The glands were fixed in Zenker's fluid, imbedded in paraffin, sectioned, and stained with Mallory's phloxine-methylene blue. Each gland was carefully compared with the other of the pair, with the remaining breasts of the same animal, with those from the other cats in the experimental groups, and, finally, with breasts removed from normal cats in corresponding stages of gestation.

Results. A. *The effect of sympathectomy on gestation and parturition.* Seven animals were pregnant during the period beginning 3 to 4 months after completion of the operations (table 1). One of these was pregnant at the time of operation. Four of the 7 animals belonged to the group with bilateral lumbosacral sympathectomy, and in 2 of these 4, one adrenal was removed and the other denervated. Only one of this group of 4 cats (A1)

carried its kittens to term. Its gestation period was normal, the breasts hypertrophied normally, and nothing unusual was observed in the postpartum growth and development of the litter. The other 3 animals

TABLE 1

*Summary of experimental animals which were successfully mated after operation*

| CAT NUMBER | OPERATION | DATE | FIRST PERIOD OF OBSERVATION | | SECOND PERIOD OF OBSERVATION | | REMARKS |
|---|---|---|---|---|---|---|---|
| | | | Date mated | Result | Date mated | Result | |
| A1 | Lumbosacral sympathectomy | 3- 9-36 | 5-22-36 | Normal litter of 3 kittens, born 7-22-36 | 3-20-37 | Ruptured uterus, 5-29-37 | Normal maternal behavior with first pregnancy |
| A2 | Lumbosacral sympathectomy | 3-18-36 | 7-16-36 | Aborted, 8-27-36 | 2- 4-37 | Litter of 4 kittens; 3 living, 1 dead; 4-5-37 | Normal maternal behavior |
| A3 | Lumbosacral sympathectomy | 5-14-36 | | | 3- 7-37 | Litter of 3 kittens; 1 living, 2 dead; 5-7-37 | Mother showed no interest in living kitten |
| B1 | Lumbosacral sympathectomy. Excision of right adrenal. Denervation of left adrenal | 3-30-36 | Pregnant at time of operation | Aborted, 5-25-36 (4 stillbirths) | | | |
| B3 | Lumbosacral sympathectomy. Excision of right adrenal. Denervation of left adrenal | 4- 2-36 | 5-22-36 | Aborted, 7-11-36 | | | |
| C1 | Excision of both stellate ganglia | 3-24-36 | 5-22-36 | Aborted, 7-12-36 | 1-26-37 | Litter of 6 normal kittens, 4-2-37 | Normal maternal behavior |
| C3 | Excision of both stellate ganglia | 5-16-36 | | | 3-14-37 | Litter of 5 normal kittens, 5-17-37 | Normal maternal behavior |
| D1 | Excision of entire left sympathetic chain. Right adrenal removed | 4- 5-36 | | | 3- 9-37 | Litter of 3 stillbirths, 5-9-37; 1 living kitten, 5-10-37 | Mother neglected kitten, which lived 4 days |
| D4 | Excision of entire left sympathetic chain. Right adrenal removed | 5-23-36 | Pregnant at time of operation | Aborted, 6-15-36 (5 stillbirths) | | | |
| E3 | Total sympathectomy in two stages | 4-25-36; 5-17-36 | 5-22-36 | Aborted, 6-27-36 (4 stillbirths) | 1-26-37 | Litter of 4 kittens; 3 living, 1 dead; 4-2-37 | Mother neglected kittens, which died next day |

aborted during the 5th and 6th weeks of pregnancy. After the abortions, kittens from other litters were permitted to suckle the breasts, but no lactation followed and the glands underwent rapid involution.

The remaining three of the seven pregnancies studied during this period

of observation were in animals belonging each to a different group. One (C1) had had both stellate ganglia removed, another (D4) had had a unilateral sympathectomy, and the third (E3) had been totally sympathectomized. All three aborted by the 5th or 6th week of gestation (one, D4, was pregnant at the time of operation, cf. table 1). The breast development was again comparable with that of normal animals in the same stage of pregnancy, and there was no lactation, but a rapid involution after the abortion.

The second period of observation began 10 to 12 months after the operations. Seven cats became pregnant (cf. table 1). There were no abortions. Three of the animals (A1, A2, A3) had had bilateral abdominal sympathectomy. One of them (A2) had aborted during its pregnancy of the first observation period. This cat gave birth to a litter of 4, one of which was stillborn. Gestation and parturition were otherwise normal. The breasts developed normally and the 3 living young were nourished. The second cat (A3) had not been pregnant during the experimental period of the preceding year. The pregnancy went to term and a litter of 3 was born. Two were stillbirths, the third died the next day, in spite of an apparently abundant supply of milk in normally developed breasts. The third cat of this group (A1) is the most interesting of the entire series. It had been pregnant the previous year, and, as described above, nothing abnormal was observed at that time. With the second pregnancy, however, parturition was at least a week overdue; the cat was inspected daily, but did not deliver. Finally, rapidly increasing abdominal distention set in, and the cat became moribund although it had looked well when seen 6 hours previously. The animal was operated upon and 4 fetuses were found lying free in the abdominal cavity. Nothing abnormal was detected in the uterine cornua, but the body of the uterus was ruptured between the two horns. No dilatation was observed in the lower end of the uterus or the vagina. Two of the fetuses were approximately twice the expected size of kittens at birth.

Rupture of the uterus in cats is extraordinary and becomes particularly significant in this case when considered together with the histological examination of the breasts removed during the 6th week of pregnancy, as will be described below. Gross examination of the mammary glands during the pregnancy had revealed no unusual difference between any of them.

From 2 (C1, C3) of the other 4 cats that became pregnant 10 to 12 months after the operations the stellate ganglia had been removed. Both pregnancies were entirely normal. Mammary development and lactation were not unusual. One of the cats had been pregnant and aborted during the observation period of the previous year.

One of the remaining 2 cats (D1) had had a unilateral sympathectomy.

It had not become pregnant during the first mating period following the operation. The pregnancy went to term and 3 stillbirths occurred on one day, followed the next day by the birth of a living kitten. This delay in the completion of parturition is not common. Bacq (1932) observed a similar phenomenon in the rat after sympathectomy. Markee and Hinsey (1935) reported a cat in which the right horn (denervated) of the uterus delivered its two fetuses, presumably after a normal gestation period, 13 days before the left (normally innervated). This phenomenon, however, was interpreted as an instance of superfetation and not as prolonged parturition.

The living kitten from animal D1 was permitted to nurse, but was otherwise neglected by the mother. It died on the 4th day, although there seemed to have been an adequate supply of milk in the mother's breasts. They were all well developed and no difference could be detected on the two sides.

The totally sympathectomized cat (E3) which had aborted its first pregnancy after the operation again became pregnant and went to term. Mammary gland development was normal. A litter of 1 dead and 3 living kittens was born with no abnormality associated with parturition. The living kittens all died the day after delivery and the mother's breasts underwent rapid involution.

B. *The effect of sympathectomy on maternal behavior.* No difference could be detected in the behavior of the completely sympathectomized, partially sympathectomized, and normal cats during gestation. The behavior of 7 cats was studied after delivery at term. The totally sympathectomized cat made no attempt to clean its 3 living kittens or otherwise care for them during the day that they lived. Similarly, the unilaterally sympathectomized cat completely neglected its kitten except that suckling was permitted. The 2 cats from which both stellate ganglia had been removed took good care of their kittens. The young were kept clean, were allowed to suckle, and were carefully watched over. The same maternal behavior was seen in 2 of the abdominally sympathectomized cats that had normal young. One of the cats with abdominal sympathectomy, however, neglected its 1 living kitten of a litter of 3.

C. *The effect of sympathectomy on the histological structure of the mammary gland during gestation and after parturition.* With one exception, total and partial sympathectomy were found to have no detectable influence on the histological appearance of the mammary glands during gestation and lactation. No difference was seen between the two glands of each pair, between pairs from the different animals of the experimental group, or between these and glands from normal animals in corresponding stages of gestation.

The only positive changes that could be attributed to sympathetic

denervation of the breasts were obtained in the abdominally sympathectomized animal that went beyond term, failed to deliver, and suffered rupture of its uterus. Figure 1 shows the striking difference between a thoracic and abdominal breast excised during the 6th week of pregnancy. The abdominal breast was hypoplastic; the thoracic breast showed evidence of marked activity, although normally the abdominal breasts are usually better developed than the thoracic. The acini in the thoracic breast were large and had a diameter of 111.0 $\mu$ (average), while the acini of the abdominal breast, measured only 38.0 $\mu$ (average). In the well-developed gland, the acinar cells were columnar but small and there was a scarcity of interlobular connective tissue. The ducts were large and lined with columnar cells. In the hypoplastic abdominal breast, the acinar elements were few in number and consisted of relatively large

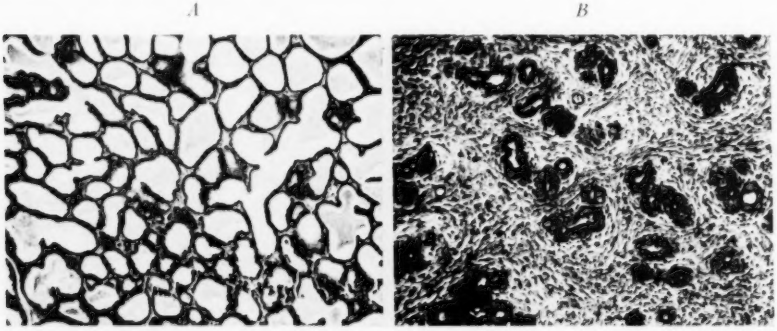


Fig. 1. Photomicrographs of a section of highest thoracic breast (*A*) and a mid-abdominal breast (*B*) from cat A1. Breasts removed during 6th week of pregnancy one year after bilateral lumbosacral sympathectomy. Magnification: medium high.

cuboidal cells. There was an abundance of interlobular connective tissue. The ducts were few and lined with cuboidal epithelium.

Additional observations were made on a dog from which the entire left paravertebral sympathetic chain and the right sympathetic chain from the diaphragm to the pelvis had been removed. Three weeks after completion of the operations the animal became pregnant, and after a normal gestation period gave birth to five normal pups. No abnormality in gestation or parturition was observed. The mammary glands developed normally; there was no disturbance in lactation; and the pups gained weight rapidly. There was nothing unusual in the maternal behavior of the animal.

Discussion. The incidence of abortions and stillbirths in cats is not known. Observations on cats living in a laboratory environment, however, would indicate that it is low. In the experimental animals reported above, there were 6 abortions in the first 7 of a total of 14 pregnancies.

Of the 8 that went to term, stillbirths were observed in 4. It is of interest that abortions all occurred among the pregnancies of the first period of observation (3 to 4 months after the completion of the operations). Stillbirths, on the other hand, occurred only in the pregnancies of the second period (10 to 12 months after the operations).

The animals used in these experiments were all in excellent condition after the operations. They were kept on an adequate diet and in rooms instead of in cages. Indeed, the fact that they came into heat is an index of their good general health. The abortions and stillbirths reported above, then, may reasonably be attributed to the specific effect of denervation of the internal generative organs. True, no histological changes can be detected in these structures after denervation (Clark, 1933; Hinsey and Markee, 1935), but whether or not there is any disturbance in function is still problematical. If a functional impairment occurs in the uterus after sympathetic denervation, comparable to that demonstrated for the epididymis of the guinea pig (Simeone, 1933), union of the germ cells may be delayed. These cells are very sensitive to ageing (cf. Young and Simeone, 1930). Mall's work (1908) suggests that defective germplasm is of fundamental importance in the etiology of abortions. Recently, Young and Blandau (1936) reported a striking increase in the incidence of abortions from prolonging the postovulatory age of ova by only a few hours. In cats B1 and D4 (table 1), sympathectomy exerted its effects on the already implanted ovum.

The fact that there were no abortions during the second period of observation a year after the operations suggests that some function may have been restored to the internal genitalia, possibly from regeneration of preganglionic fibers to the outlying ganglia. The high incidence of stillbirths, however, would indicate that the return of function was not complete.

One of the abortions occurred in an animal (C1, table 1) in which the innervation of the abdominal viscera was intact. There is no obvious explanation for this observation. The cat was mated with the same male as cat A1, which delivered a normal litter of 3 kittens. To be sure, the abortion may have been purely coincidental.

The observations on the abnormal maternal behavior of 4 of the 7 cats agree with those of Cannon (1930). Afferent impulses from the uterus may have widespread effects within the organism. Selye (1934b) showed that, in rats, lactation follows emptying of the uterus by Caesarian section, but not if the uterine cavity is refilled with paraffin. Sensory impulses from the uterus, therefore, may well play an important rôle in determining the complex behavior pattern of the maternal instinct. Why this is not lost in all cases of sympathetic denervation of the abdominal organs is not clear.

Sympathetic denervation of the mammary glands caused definite variation from normal functional activity, recognizable histologically, in only 1 out of 7 pregnancies that went to term. Careful observations on the character and quantity of milk secreted (cf. Cannon and Bright, 1931) were not made. They might have revealed functional abnormalities even in those cases in which mammary gland development appeared normal histologically. It may be significant that the cat which showed underdevelopment histologically suffered rupture of the uterus in the same pregnancy, probably more than a mere coincidence. Of further interest is the fact, agreeing with the observations of Cannon and Bright (1931) for the cat and of Bacq (1932) for the rat, that the disturbances in lactation were not encountered with the first litter, but with the second, after the operations. The changes leading to impairment of lactation apparently do not develop immediately after denervation but only after some time.

It is important to note that the disturbance in lactation occurs not in all animals, but only in relatively few. The fact cannot be attributed to incomplete denervation or to regeneration because the first pregnancies after denervation (cf. Cannon and Bright, 1931; Bacq, 1932) are followed by normal lactation.

The impairment of lactation following sympathectomy is probably not due to a disturbance in the endocrine balance (cf. Bacq, 1932) because the deficiency appears to be limited to the denervated gland. The activity of the mammary glands is in part conditioned by their blood supply. Release of the local blood vessels from central vasomotor control may, therefore, play a part in the disturbed function of these glands after sympathectomy. One would expect, however, that this influence would be exerted on the first as well as on subsequent litters after the operations.

The possibility remains that sympathetic nerve impulses have a specific influence on the mammary gland cells. Clark (1933) detected no changes in the mammary glands attributable to sympathetic denervation, and Ernst (1929) likewise found no histological changes if regional sympathectomy alone was done, though atrophy occurred if corresponding intercostal nerves were severed. Their studies were done on resting glands. It is possible that sympathetic denervation may induce a functional change in the secretory cells which develops slowly and in some instances is sufficient to prevent the usual response of these cells to the hormones of pregnancy. Such a change might be expressed as an increased threshold of the mammary glands to the circulating hormones, an effect, however, which would be in contrast to the usual effect of denervation on the responsiveness of autonomic effectors (cf. Cannon and Rosenblueth, 1937). The inconstancy of the impairment of lactation after sympathetic denervation might then be associated with variation in the concentration of circulating hormones in different animals.

SUMMARY

1. Observations are reported on gestation, parturition and lactation in totally and partially sympathectomized cats shortly after completion of the surgical procedures and a year later (table 1).

2. The incidence of abortions is high in animals that become pregnant shortly after sympathetic denervation of the internal genitalia. The incidence of stillbirths is high in animals that become pregnant long after sympathectomy (table 1).

3. Rupture of the uterus and failure of development of the abdominal breasts are reported for one cat after abdominal sympathectomy (table 1; fig. 1).

4. The rôle of the sympathetic nervous system in reproduction and lactation is discussed (pp. 664–666).

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# FACTORS DETERMINING VOLUNTARY INGESTION OF WATER IN NORMALS AND IN INDIVIDUALS WITH MAXIMUM DIABETES INSIPIDUS

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Received for publication January 24, 1938

The rôle played by the voluntary ingestion of water, or by thirst, has not received much attention in studies on water metabolism. Most of the interest has been centered on absorption, distribution, and elimination of definite amounts of administered water. It is a commonly observed fact that animals and humans voluntarily drink water when they become dehydrated, when there is apparently a real need for water. However, much of the ingestion of water appears to be entirely fortuitous with little relation to actual needs.

In a previous study (Richter and Brailey, 1929) it was shown that the amount of voluntarily ingested water in normal rats at various ages is directly proportional to the surface area of the animal. In other words, it was shown that it is dependent on the metabolic rate.

In the present study, the general applicability of this principle to cats, dogs, monkeys, humans, as well as to rats, was tested.

The voluntary ingestion of water of animals with maximum diabetes insipidus was studied to determine whether it is related to surface area or to some other factor.

METHODS. The method of measuring water intake of the rat has been described in detail in a previous paper (Richter and Brailey, 1929). Water was offered in graduated, inverted bottles containing 100 cc. It was changed at least every other day and in some instances every day. Readings were made every day at noon.

Data on the water intake of normal rats were taken from observations made on 51 animals during the period from 30 to 160 days of life (Richter and Brailey, 1929). For purposes of comparison, the water content of the food, and water of oxidation have been added to the figures for the actual water ingested. Unless these additional sources were taken into account, it would be incorrect to compare the water intake of a rat, in which they supply 25 per cent of the total intake, with that of a cat, in which they may represent 70 per cent of the intake.

Records of the maximum water intake of rats with diabetes insipidus were obtained from 2 groups of animals, one group of 530 animals with chronic symptoms observed over long periods of time, and one group of 240 with acute symptoms observed for 10 to 20 hours postoperatively. The diabetes insipidus was produced in both groups of animals by any one of the 5 following operations: total or partial hypophysectomy, posterior lobectomy, stalk section, or a brain stem stab through the stalk. Only the records showing the maximum intake were used for the present purposes.

For reasons which will be disclosed below, records were taken also on forced water intake and output in 5 groups of 8 rats each, which were given hourly 2.5, 5, 10, 15, and 20 cc. of water respectively by stomach tube over an 8 hour period. The rats were kept separately in small cages, $4\frac{1}{2}$ x $4\frac{1}{2}$ x 8 inches, with wire mesh bottoms to catch the feces but not the urine. The urine was collected in a graduated cylinder by means of an inclined trough. Records were made hourly of the urine output. The animals were weighed at the beginning and end of the experiment. No food was given.

Records of the normal maximum intake of cats, monkeys, dogs, and man were obtained largely from observations made by others. The data on the intake of normal cats were obtained from records provided by Dr. Clinton M. Woolsey and Dr. Chandler Brooks (personal communication). The average normal intake was 321 cc. for fifty animals, averaging 4.49 kgm. in weight. The highest intake record of cats with diabetes insipidus was reported by Ranson (1936). One animal, in which the supraoptic tracts had been destroyed by the Horsley-Clarke apparatus, weighed 2.5 kgm. and had an intake of 1,213 cc.

The average daily intake of normal monkeys was taken from a paper by Krohn and Zuckerman (1937), who reported an intake of 397 cc. for an animal weighing 4.9 kgm. The highest intake record for monkeys with diabetes insipidus was reported by Dr. S. W. Ranson (personal communication). His animal, in which the supraoptic tracts had been destroyed by the Horsley-Clarke apparatus, weighed 2.75 kgm. and had an intake of 1,335 cc.

Measurements of the normal intake of dogs were made in this laboratory. Daily readings were taken on 8 animals over periods varying from 1 to 3 months. The water was offered in small pans firmly attached to the side of the cage to prevent spilling. Allowance was made for evaporation. The average weight of these 8 animals was 10.7 kgm. and the average intake 603 cc. The highest intake in dogs with diabetes insipidus was reported by Dr. Stephen Maddock (personal communication). His animal, in which a silver clip had been applied to the pituitary stalk, weighed 13.8 kgm. and had an intake of 9,220 cc. per day. Other high

intake records have been reported. Unfortunately, most authors have reported high water exchange in terms of output rather than intake; however, in instances where both are given, they are found to run closely parallel. Teel (1929) obtained a 24 hour output of 7,300 cc. of urine by injecting an 18 kgm. dog with an extract containing the growth promoting principle. Dr. R. T. Bellows and Dr. W. P. Van Wagenen (personal communication) reported outputs of 7,500 cc. and 8,800 cc. in dogs weighing 17.5 and 18.8 kgm. respectively. Bailey and Bremer (1921), by temporal puncture in an adult male dog weighing 12.9 kgm., produced a diuresis of 3,800 cc. Mahoney and Sheehan (1936), using a silver clip, occluded the pituitary stalk of a mongrel female puppy weighing 3 kgm. and obtained a urine output of 1,500 cc.

The estimated normal intake of 2,400 cc. in an average man (weight, 65 kgm.) was taken from data compiled by Best and Taylor (1937). The highest available records for humans with diabetes insipidus were reported by Whitehead and Darley, Trousseau, and Blumgart. Trousseau (1870) reported the classical case of a young man drinking 41 liters in 24 hours and voiding from 37 to 43 liters in the same space of time. Whitehead and Darley (1931) cited the case of a fourteen-year old boy, 37.7 kgm., who, following encephalitis, had a 24 hour output of 38 liters against an intake of 35 liters. Dr. Herrman L. Blumgart (personal communication) described a case on the surgical service of the Peter Bent Brigham Hospital, of a male, seventeen years of age, who 2 years prior to hospital admission had a pneumococcus meningitis and encephalitis. Five weeks following this, the patient noted polydypsia and polyuria, which gradually increased. His fluid intake was 41 liters with a urine output of 40 liters. These symptoms were associated with evidence of cerebral pathology. Postmortem showed fibrosis in the region of the pituitary gland which was largely replaced by cysts.

RESULTS. *Normal water intake.* The records of the normal intake for different animals are shown in columns 5, 8, and 11 in table 1. It will be seen that the intake is a function of body surface rather than of body weight in all of the animals and humans. The average daily intake per square meter body surface varied only between 1,050 and 1,238, while the intake per kilogram of body weight varied from 37 to 157 cc.

Evidence was thus obtained to show that the voluntary intake in normal animals is dependent on body surface and hence must indirectly be dependent on the metabolic rate. It has been shown previously (Richter and Brailey, 1929) that for rats varying from 50 to 325 grams in weight, water intake is a function of surface area rather than of body weight. In agreement with this finding is the fact that in rats increased metabolism, produced by the ingestion of large quantities of thyroid extract, is associated with increased water intake (Richter, 1933).

*Maximum water intake of diabetes insipidus.* The highest intake record observed in our rats with chronic diabetes insipidus is shown in figure 1. A stab lesion made with a scalpel at the anterior margin of the pituitary gland produced an increase in daily water intake from a level of 20 cc. to a level of over 200 cc. The high intake level was maintained up to the time the animal was killed, about 6 months postoperatively. This record

TABLE 1

*Water intake in normals and in individuals with maximum diabetes insipidus*

| ANIMAL | AUTHOR | DATE | WEIGHT | ACTUAL WATER INTAKE | | | INTAKE PER KILOGRAM OF BODY WEIGHT | | | INTAKE PER SQUARE METER OF BODY SURFACE | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Normal | Maximum diabetes insipidus | Ratio | Normal | Maximum diabetes insipidus | Ratio | Normal | Maximum diabetes insipidus | Ratio |
| | | | *kgm.* | *cc.* | *cc.* | | *cc.* | *cc.* | | *cc.* | *cc.* | |
| Rat | Richter | | 0.225 | 35.3 | | | 157 | | | 1,050 | | |
| | Acute | | 0.217 | | 232 | 7:1 | | 1,075 | | | 7,100 | |
| | Chronic | | 0.160 | | 192 | 5.5:1 | | 1,195 | 7:1 | | 7,150 | 7:1 |
| | Forced diuresis | | 0.247 | | 249 | 7:1 | | 1,008 | | | 7,440 | |
| Cat | Brooks and Woolsey | * | 4.49 | 321 | | 4:1 | 71.5 | | | 1,055 | | 5.5:1 |
| | Ranson | 1936 | 2.5 | | 1,213 | | | 485 | 7:1 | | 5,860 | |
| Monkey | Krohn and Zuckerman | 1937 | 4.9 | 397 | | | 81 | | | 1,158 | | |
| | Ranson | * | 2.75 | | 1,335 | 3.5:1 | | 485 | 6:1 | | 5,710 | 5:1 |
| Dog | Richter | | 10.7 | 603 | | 15:1 | 56 | | 12:1 | 1,238 | | 11:1 |
| | Maddock | * | 13.8 | | 9,220 | | | 666 | | | 14,150 | |
| Man | Best and Taylor | 1937 | 65 | 2,400 | | | 37 | | | 1,210 | | |
| | Whitehead and Darley | 1931 | 37.7 | | 35,600 | 15:1 | | 945 | 25:1 | | 26,600 | |
| | Blumgart | * | 53.8 | | 41,600 | 17:1 | | 778 | 21:1 | | 24,800 | 22:1 |
| | Trousseau | 1870 | 50† | | 41,600 | 17:1 | | 833 | 23:1 | | 25,900 | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |

* Personal communication.

† Estimated.

These figures take into account water content of food and water of oxidation.

is reported in table 1. The animal weighed 160 grams. Its daily intake averaged 192 cc. or 1,195 cc. per kilogram body weight or 7,150 cc. per square meter body surface.

The average maximum daily intake of rats with acute diabetes insipidus was taken from records of 7 animals showing the highest intake. The record of one of these animals is presented in figure 2A. The averages

for the 7 are recorded in table 1. The average body weight was 217 grams. The daily intake was 232 cc. or 1,075 cc. per kilogram body weight or 7,100 cc. per square meter body surface. Thus in columns 9 and 12 it will be seen that the averages for the rats with acute and chronic diabetes insipidus were approximately the same.

The records for the other animals and humans showing maximum diabetes insipidus are listed in table 1. It will be seen that the voluntary water intake in maximum diabetes insipidus is a function of body weight rather than of body surface. For the rat the maximum average daily intake per kilogram body weight was approximately 1,000 cc. and for man 945 cc. (Whitehead and Darley, 1931). The fact that the records for

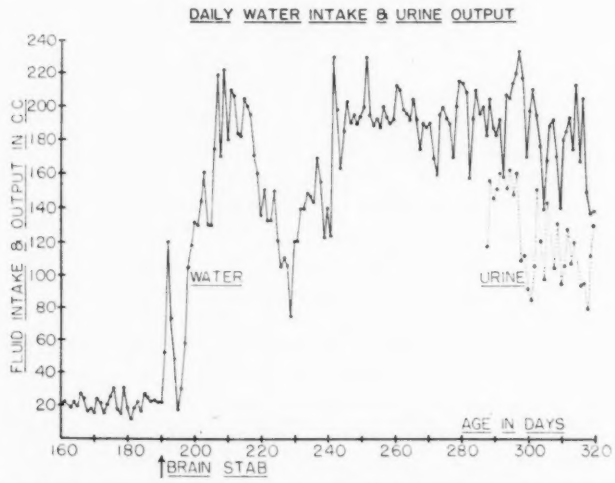


**Fig. 1. Maximum water intake in a rat with diabetes inspidus produced by a brain stem stab.**

the cat, the monkey, and the dog were slightly lower probably indicates that in these animals a maximum diabetes insipidus had not been produced. The lack of any relation to body surface may be clearly seen in column 12, which shows a variation from approximately 7,000 cc. per square meter body surface daily for the rat to 26,000 cc. for man.

*Maximum forced water diuresis in the rat.* It occurred to us that it might be of interest to compare the maximum diuresis of diabetes insipidus with the maximum forced water diuresis in order to determine how nearly a maximum level had actually been reached in the diabetic animals. The conditions of these experiments were the same as in the experiments on acute diabetes insipidus. Rats were kept separately in small cages

provided with means for collecting the urine. Three typical records of animals receiving 5, 10, and 20 cc. of water per hour over 8 hour periods are shown in figure 2 (B, C, and D). Water was introduced by stomach tube. With injections of 5 cc. per hour, the output was approximately the same as the intake; with 20 cc. it was markedly lower, indicating that the eliminating mechanism was not able to keep up with the water intake. The results of these experiments are summarized in figure 3 (A and B), which shows the urine output of 5 groups of animals receiving 2.5, 5, 10, 15, and 20 cc. of water per hour respectively. In figure 3 it will be seen that the maximum output was about 1,000 cc. per day per kilogram body

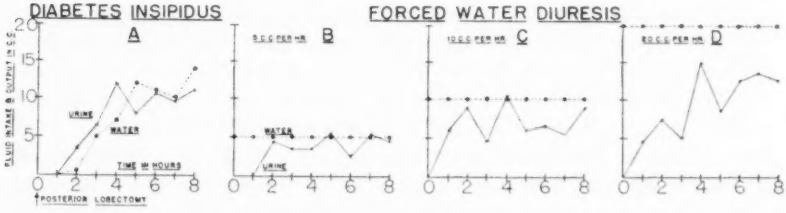


Fig. 2. Maximum water intake and output in diabetes insipidus and in forced diuresis in rats. A, intake and output in diabetes insipidus produced by posterior lobectomy. B, C, D, output produced by giving 5, 10, and 20 cc. of water per hour by stomach tube.

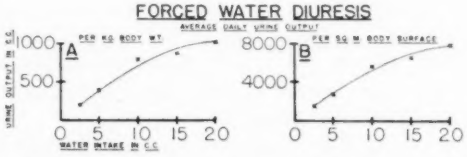


Fig. 3. Water intake and output in forced diuresis in rats compared on the basis of body surface and body weight.

weight, and about 8,000 cc. per square meter body surface per day. These figures are practically identical with the records for acute and chronic diabetes insipidus shown in table 1. It would seem likely that the maximum output is determined by the total fluid capacity of all the cellular spaces of the body.

*Ratio of normal intake to intake of maximum diabetes insipidus.* The data gathered in table 1 made possible a study of the ratios of the intake of normals to the intake of animals with maximum diabetes insipidus. These ratios are presented in columns 7, 10, and 13. It will be seen that for any one animal they are approximately the same, whether calculated for actual water intake, per kilogram body weight, or per square meter

body surface. They differ very widely, however, from one species to another, being approximately 7:1 in the rat and as high as 25:1 in man.

DISCUSSION. On the basis of the present results and the knowledge of the action of the antidiuretic pituitrin in normal and pathological states, it is possible to discuss in more detail the rôle played by thirst or voluntary ingestion of water in maintaining normal water balance or homeostasis.

We know from the excellent experiments of Klisiecke, Pickford, Rothschild, and Verney (1933), and Burgess, Harvey, and Marshall (1933) and of Gilman and Goodman (1937) that the posterior lobe secretion in normal animals plays an important part in the maintenance of the water balance. A decrease in the supply of water produces dehydration, which in turn causes a greater secretion of pituitrin, thus reducing the normal output and conserving fluid. When, on the other hand, the water intake is forcibly increased, the secretion of pituitrin is decreased and more water is lost. In the normal animal with free access to water, the water balance is taken care of by the animal's voluntary ingestion of water, which we have shown is dependent on metabolism. Although in normal animals self-regulation of water plays a part in the maintenance of the water balance, it is clear that the secretion of pituitrin probably plays the more important part.

In animals with total removal of the posterior lobe, the conditions are considerably altered; in this case, the maintenance of the water balance is dependent entirely on the voluntary ingestion of water. There seems to be little doubt that the removal of the posterior lobe produces a diuresis and, as a result of this loss of urine, an increased thirst (Richter, 1935; Richter and Eckert, 1935). The rôle played by metabolism on the water intake of partially and totally hypophysectomized animals has been described elsewhere (Richter, 1934).

The fact that a maximum diuresis obtained by posterior lobectomy in rats was equal to that obtained by forced water diuresis must indicate that after the removal of the posterior lobe the kidney activity goes on entirely unchecked until it reaches its full capacity.

Since both the maximum intake and the size of the kidney have been shown to be dependent on body weight, they may have a direct relationship to each other. In other words, the maximum intake may be determined by the maximum capacity of the kidneys.

## SUMMARY

1. The voluntary water intake of normal animals and humans was found to be a function of surface area rather than of body weight, and hence must be a function of metabolism.

2. The average daily water intake per square meter body surface varied from 1,050 cc. to 1,238 cc. in rats, cats, dogs, monkeys, and humans, averaging 1,142 cc.

3. The maximum voluntary water intake in animals with diabetes insipidus was found to be a function of body weight rather than of body surface.

4. That a maximum output level had been obtained in rats with diabetes insipidus was shown by the fact that it was not possible to produce a further increase by forcing large quantities of water.

5. The average daily intake per kilogram body weight was approximately 1,000 cc. for rats and humans, and slightly lower in cats, dogs, and monkeys, due undoubtedly to the fact that in these animals a maximum diuresis has not yet been obtained because of the incomplete removal or inactivation of posterior lobe tissue.

6. The ratio of the water intake of animals with diabetes insipidus to the intake of normals was found to be approximately the same in each species, whether based on the actual intake or on the intake per kilogram body weight or per square meter body surface. However, the ratio varied widely from one species to another. Thus in the rat it was 7:1 and in humans 25:1.

7. The factors governing the maximum diuresis in any one species were not definitely determined. It was suggested that the level of the maximum intake of diabetes insipidus might be determined by the maximum capacity of the kidney or by the maximum capacity of all the cellular spaces of the body, both of which would be functions of body weight.

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# THE ACTION OF CYANIDE AND OF OXYGEN LACK ON GLOMERULAR FUNCTION IN THE PERFUSED FROG'S KIDNEY[1]

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Received for publication January 26, 1938

Observations by Detering (1), Höber and Mackuth (2) and other former workers (3) in the Kiel laboratory have shown that urine formation by the perfused frog's kidney is decreased by the addition of cyanide or phenyl urethane to the arterial perfusion fluid or by the absence of oxygen from it. The fact that the rate of total arterial perfusion flow was not correspondingly diminished seemed to preclude decrease in glomerular flow and glomerular capillary pressure as explanatory of the oliguria; hence the effect was ascribed to suppression of a secretory process in the glomerular membrane. The conclusion was drawn that glomerular urine is formed by secretion, not by filtration (4).

Detailed study of the effects of the agencies mentioned upon the perfused vessels was not made except in the later work of Brühl (3) who undertook to observe glomerular capillary flow by direct microscopic observation, beef erythrocytes having been added to the arterial perfusion fluid to render glomerular flow visible. He was unable to see marked change in number of glomeruli perfused and reported his results as confirmatory of the glomerular secretion hypothesis.

We have repeated and to some extent amplified these experiments. The chief outcome is the conclusion that the rate of total arterial perfusion flow is a misleading index of *glomerular* perfusion. The arterial perfusion fluid finds access not only to the true renal vessels which supply the glomeruli but also to a significant number of extra-renal vessels which do not. Evidence will be outlined which shows that the true renal arteries and arterioles may be constricted by cyanide or asphyxia while the extra-renal arterial vessels dilate. The net result may be restriction of glomerular perfusion, with consequent decrease in glomerular capillary pressure and oliguria, associated with no corresponding restriction of total arterial perfusion flow. We therefore conclude that when the possibility of mutually compensating actions of CN or asphyxia upon renal and extra-

[1] The expenses of this work were defrayed in large part from a grant by the Commonwealth Fund.

renal vessels is taken into account, the oliguria observed by Höber and Mackuth can be explained as the result of decrease in glomerular filtration.

*Repetitions of the experiments of Höber and Mackuth. Perfusion technique.* Both male and female specimens of *R. catesbiana* and *R. pipiens* were used; *R. esculenta* were not obtainable. The brain and cord were pithed, the abdomen quickly opened and a cannula, pointing toward the heart, inserted into the aorta between its bifurcation and the lowest of the renal arteries. This cannula was at once connected with the arterial perfusion bottle; in consequence vigorous circulation through the kidneys was maintained during the rest of the preparations. Cannulas were inserted into the anterior abdominal vein for perfusion of the renal portal vessels, into the vena cava, and into one or both ureters. The aorta was ligated immediately posterior to the origin of the coeliaco-mesenteric artery. The ureteral cannulas were connected with horizontally placed 1 cc. pipettes, graduated in 1/100's. The perfusion fluid, "Ringer's solution," was made according to the formula of Barkan, Broemser and Hahn (5) from glass distilled water and the purest obtainable chemicals.[2] Unless otherwise stated it was saturated with $O_2$ containing 1.5 per cent $CO_2$. Its pH was always within the range 7.4 to 7.8.

Perfusion pressures in many instances were the same as those usually chosen by the Kiel experimenters (aortic 24: renal portal, 12 cm.). Not infrequently however it was necessary to adjust the pressures differently in order to make urine rates fall within convenient limits of measurement. We are unable to agree with Brühl (3) that urine flow is not altered by changes in arterial perfusion pressure.

The perfusion bottles in the first group of experiments consisted of inverted 50-cc. burettes arranged to function as Marriott bottles. Y-tubes and clamps made it possible to refill a burette without interrupting the renal perfusion; the gas which replaced the fluid which flowed from the burette was the $O_2$-$CO_2$ mixture with which the perfusion fluid had been saturated. In the later experiments 300-cc. Wasserman burettes with 5 cc. graduations and Marriott stoppers were similarly used.

Chlorides in perfusion fluids and urines were determined by the Whitehorn method (6); creatinine by the Folin method (7); glucose and inulin either by the Shaffer-Somogyi (8) or the Hagedorn-Jensen method (9); pH of perfusion fluids either colorimetrically or with the glass electrode.

RESULTS. A series of 20 experiments was made by Kempton in 1934–1935 in which the effect of M/1500 KCN in the arterial perfusion fluid was tested in large female specimens of *R. pipiens*. In 10 the perfusion fluid contained beef erythrocytes prepared according to Brühl; in 10 it was

[2] In the majority of our experiments glycine was added to the perfusion fluid to make 0.025 per cent. This was done to conform to the Höber technique. Its omission did not seem to influence the course of an experiment.

Ringer without erythrocytes. Perfusion pressures were aortic, 24; renal portal, 12 cm. water. In 12 the average rate of urine flow during the KCN period was higher than during the preceding control period; in 6 it remained practically unchanged; in 2 it decreased, but in these the decrease in urine rate was so strikingly parallel with concurrent decrease in rate of aortic perfusion flow as to make it certain that constriction of vessels was the cause of the decrease in urine. The results of 3 experiments, typical of the series, are reproduced in figure 1.

Two years later another series was made with more encouraging results. To avoid change of pH in the perfusion fluid by KCN a solution of HCN, made by neutralizing N/10 NaCN with HCl to pH 7.4, was used in making

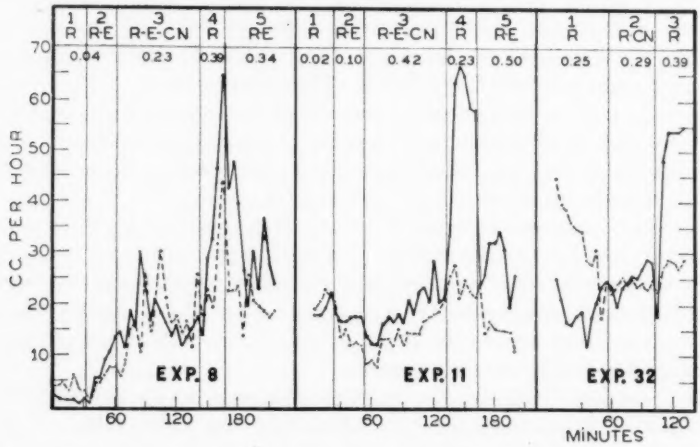


Fig. 1. *R. pipiens*, ♀ arterial perfusion with M/1500 KCN. Three representative experiments, first series. Solid line = urine (cc. per hr. × 50); broken line = arterial perfusion flow. *R* = Ringer's solution, *R-E* = erythrocyte Ringer. Figures above curves = NaCl concentration of urines.

the dilutions tested. Typical results are shown in figure 2. In 9 experiments (16 tests) with *R. pipiens*, M/1500 HCN in the arterial perfusion fluid produced the Höber effect 6 times; i.e., urine rate decreased with no corresponding decrease in arterial perfusion flow. In 9 experiments (18 tests) with *R. catesbiana*, the Höber effect was obtained 10 times. In eight experiments (18 tests) with *R. catesbiana* M/1000 HCN yielded the Höber effect 4 times. M/500 HCN, tested 12 times in 4 experiments with *R. catesbiana* and one with *R. pipiens* did not give the effect once. In two instances, one of the two kidneys of a frog responded to arterial HCN with the Höber effect, the other did not. Parallel decrease in both urine formation and arterial perfusion flow occurred in 14 tests; parallel increase in both in 11 tests; no significant change in urine flow occurred in 19 tests.

*Oxygen-lack.* Four experiments were made in which the effect of an arterial perfusion fluid, freed from $O_2$ by bubbling nitrogen through it, was tested.[3] In 2 experiments the nitrogen was purified by passage through alkaline pyrogallol and the whole frog preparation during the perfusion was enclosed in an atmosphere of $N_2$ in a gas-tight chamber (Adolph, 10). In 3 of the 4 experiments urine formation diminished when the arteries were perfused with $O_2$-free Ringer. In 2 of these there was concurrent

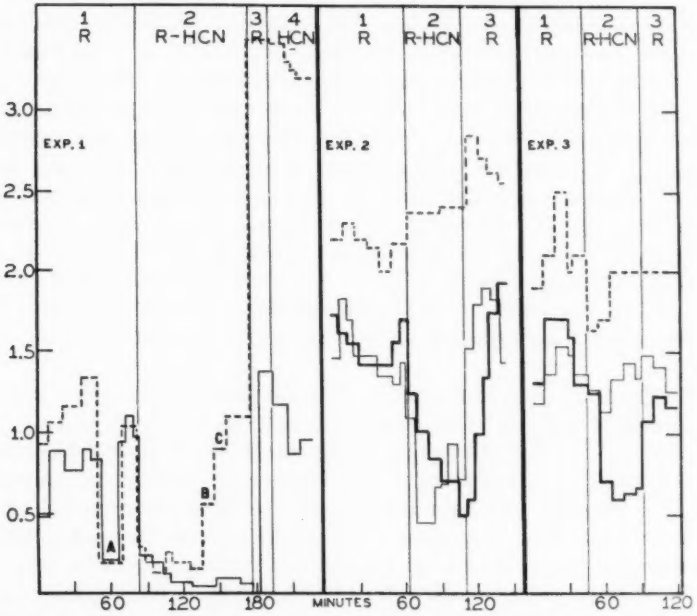


Fig. 2. Arterial perfusion with M/1500 HCN. Three representative experiments, second series. Solid lines = urine (cc. per hr.). Broken lines = arterial perfusion flow, cc. per minute. Expt. 1 *R. pipiens*, ♀. Perfusion pressures, 18:9. During period *A*, aorta was partially obstructed; at *B* and *C* arterial perfusion pressure was raised to 24 and 30 cm. Expts. 2 and 3, *R. catesbiana*, ♂.

diminution in arterial perfusion flow; hence in only one experiment (4 tests) was the Höber effect reproduced.

*Phenyl urethane.* Attempts to confirm the results of Höber and Mackuth (2) with this narcotic have almost uniformly failed. Six experiments were made in the month of October with *R. catesbiana* with concentrations of 0.005, 0.01 and 0.02 per cent phenyl urethane in the arterial perfusion

[3] In these experiments the pH of the perfusion fluids was adjusted by adding HCl in amount sufficient to neutralize one-tenth of the $NaHCO_3$ after the fluid had been saturated with $N_2$ or $O_2$.

fluid. In all, 20 tests were made. A definite Höber effect was observed three times; twice it was questionable. In 11 instances changes in urine flow and in arterial perfusion flow were parallel; in 4, results were erratic (increase in urine without change in aortic perfusion flow or decrease in aortic perfusion flow without decrease in urine).

In none of the experiments summarized above was tubule function abolished by brief renal portal perfusion with 1:10,000 $HgCl_2$, as in many of the experiments of Höber and Mackuth. There is reason to think that that procedure is not an uncomplicated means of accomplishing this result.[4] We have, however, convinced ourselves that changes in urine formation induced by CN, whether increase or decrease, are accompanied by similar changes in glomerular clearance. In several of the experiments represented by figure 1 creatinine was added to the arterial perfusion to make 10 mgm. per cent and the amount excreted in the urine specimens determined. In every case the creatinine clearance changed in parallel with urine rate. In later experiments glomerular clearances were determined by means of inulin with similar results. For example, in experiment 2 of figure 2, the inulin clearances for the 3 successive periods were 4.43, 2.38 and 3.78 cc. per hour. Adolph's experiments (10) make it certain that lessened glomerular excretion is the chief factor in urine suppression by asphyxia.

The summary given above shows that the phenomenon which we were attempting to study is by no means constant. It seems not to have been constant also in the Kiel experiments for David (11), in work with cyanide which antedated that of Detering and Höber and Mackuth, did not identify its occurrence. We have not been able to account for our failures by imperfect technique. The capacity of the kidneys in the control periods to reabsorb glucose and chloride was frequently tested by analysis of urines and found to be well retained.[5] Seasonal variations in the animals seemed not to be responsible, since failures were encountered in summer as well as in winter. We may assume that several factors are concerned, independent variations in which make the results variable. In the following pages partial identification of these is described.

*Arterial distribution of the aortic perfusion fluid.* When the vessels of the frog's kidney are perfused by way of the aorta it is obvious that other arteries than those which supply glomeruli are reached by the perfusion

[4] It was found by one of us that 3 minutes' renal portal perfusion with 1:10,000 $HgCl_2$ is sufficient to cause marked edema of the kidney and desquamation of tubule cells. The vascular and tubular damage was such that india ink added to the renal portal perfusion fluid appeared in the urine (Kempton, R. T. Anat. Rec. **64**, Suppl. No. 1, 69, 1935).

[5] For example, in 11 experiments of the first series urinary Cl in the control period was less than 0.2 per cent (as NaCl); in 4, less than 0.1 per cent. NaCl in the perfusion fluid was 0.65 per cent.

fluid. The lumbar arteries which, in *R. catesbiana*, may number as many as seven arise from the aorta at approximately the same levels as the urogenital arteries. Branches of the urogenital arteries provide direct arterial supply to the ureters (fig. 3A). Particularly conspicuous is the branch of the posterior renal artery which, passing onto the ureter, anastomoses with the peripheral distribution of intra-abdominal branches of the iliac arteries (cf. Kempton, 12). In male *R. pipiens* there are additional branches to the Mullerian ducts (fig. 3B) and in the females of both

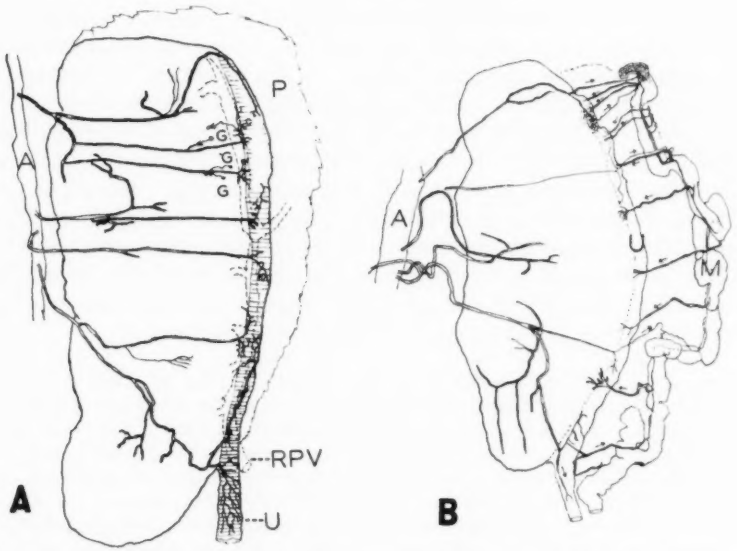


Fig. 3. "Extra-renal" branches of the urogenital arteries. A, *R. catesbiana*, ♂. B, *R. pipiens*, ♂. *A*, aorta, *U* = ureter. *RPV* = renal portal vein. *P* = peritoneum. *G* = glomeruli, *M*, Mullerian duct. No attempt had been made to include the arterial supply to glomeruli.

species to the oviducts. There is also within the substance of the kidney an unknown number of arteriae rectae.[6]

In the Höber experiments and in ours all of these arteries were accessible to the fluid delivered into the aorta. In the Höber experiments access of perfusion fluid to the vessels of the legs was prevented by mass ligatures about the thighs. This precaution did not prevent access to the posterior

[6] In such preparations as those illustrated by figure 5B, many minute vessels were judged from the character of their branchings and lack of connections with glomeruli to be arteriae and arteriolae rectae. If this judgment is correct the number of these in the frog's kidney is greater than has previously been supposed.

mesenteric artery and to the branches of the iliacs which give rise to the epigastric and recto-vesical arteries. (In our experiments these were excluded.) Hence arterial perfusion of the kidney via the aorta actually involves perfusion of two types of arteries: *a*, those which supply glomeruli and which may be called true renal arteries; and *b*, those which do not supply glomeruli and which, whether within or without the kidney, may be called extra-renal. The number and capacity of the latter is by no means negligible; more of these were perfused in the Höber preparation than in ours.

Evidence is at hand which shows that these two types of vessels differ in their reaction to CN and to $O_2$-lack; the true renal arteries constrict; the extra-renal arteries dilate or, at least, do not constrict. Consequently, change in distribution of the arterial perfusion fluid between the renal and the extra-renal arteries must result from the action of either of these agencies whereby flow through the renal arteries will decrease; through the extra-renal arteries, increase, or remain unchanged. Record of total arterial perfusion flow can not, therefore, truly represent the change which takes place in glomerular perfusion flow. Typical evidence with respect to CN is contained in the following experiment, in which the urogenital, lumbar and iliac arteries were perfused from one source, the venous return from the kidney and the leg areas being collected separately.

August 5, 1937. *R. catesbiana*, ♀, 288 grams. Arteries to oviducts tied off. Coeliaco-mesenteric and posterior mesenteric arteries ligated and gastro-intestinal tract excised. The perfusion cannula was inserted into the aorta immediately posterior to the origin of the coeliaco-mesenteric artery. Both renal portal veins were ligated close to the posterior pole of each kidney and cannulas inserted, through which to collect venous return from legs and lower abdominal muscles. A cannula was inserted into the vena cava for collecting venous return from the renal region. A cannula was inserted into each ureter. Perfusion pressure was constant at 19 cm. The results are charted in figure 4. The effect of HCN on vessels of the urogenital system was constriction; on vessels of the legs, dilatation.

Six additional experiments of this type (11 tests) were made, all on male bull frogs, 4 with M/1500, 2 with M/500 HCN. In all urine formation decreased; total perfusion flow either increased or remained approximately constant. In all but 3 tests the leg vessels dilated; in those they did not constrict. Parallelism between vena cava outflow and urine flow occurred 8 times—frequently enough to justify the conclusion that the true renal vessels are usually constricted by HCN with consequent decrease in glomerular capillary flow and pressure. In 5 additional experiments the urogenital and lumbar arteries were perfused from one bottle; the arteries of one leg from another, pressures in the two being the same. Perfusion rate through the leg was uniformly increased by M/1500 HCN; it decreased when perfusion with normal Ringer was resumed. Perfusion through

the renal area was diminished in 4 tests; not changed in 2; slightly increased in 3.

From these results it appears that with respect to cyanide the greater the number of extra-renal arteries included in the arterial perfusion system, the greater the uniformity with which the Höber effect is obtained. In such experiments as that last cited the number was greater than in those from the Kiel laboratory; in those in turn the number was greater than in our repetitions. We have only to assume that the lumbar arteries and the

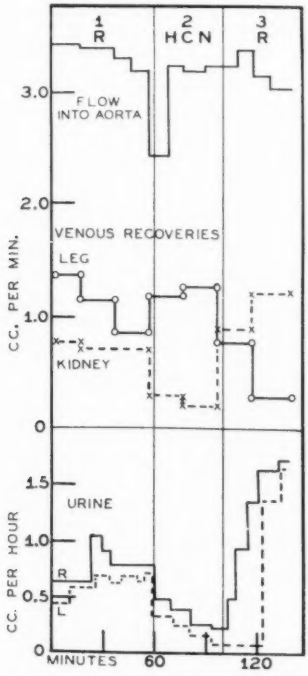


Fig. 4. *R. catesbiana*, ♀. Aortic perfusion of kidneys and legs. M/1500 HCN. Separate venous recoveries from the two regions.

branches of the urogenital arteries which do not supply glomeruli are dilated by CN, as are the arteries of the legs, while the glomerular arteries are constricted, to have an explanation of the Höber phenomenon which is wholly consistent with belief in glomerular filtration. Decrease in glomerular perfusion caused by CN is not truly revealed in measurements of total arterial perfusion flow. The seemingly unimportant modification of technique which we introduced—ligation of the aorta below the last urogenital artery and consequent exclusion of the posterior mesenteric and

first branches of the iliac arteries—was probably one of the factors in our failures to repeat the Höber results.

The above conclusion may seem to be at variance with Brühl's report that the number of visible glomeruli through which the perfusion fluid flowed did not change as a result of addition of KCN to it. We hold that changes in glomerular capillary pressure, unless extreme, can not be identified by such a method. In experiments similar to those of Brühl, we have found it impossible by watching glomeruli, either singly or in groups, to distinguish correctly pressure changes of 5 or 10 cm. within the range of 15 to 35 cm., when these were produced by raising or lowering the perfusion bottle.

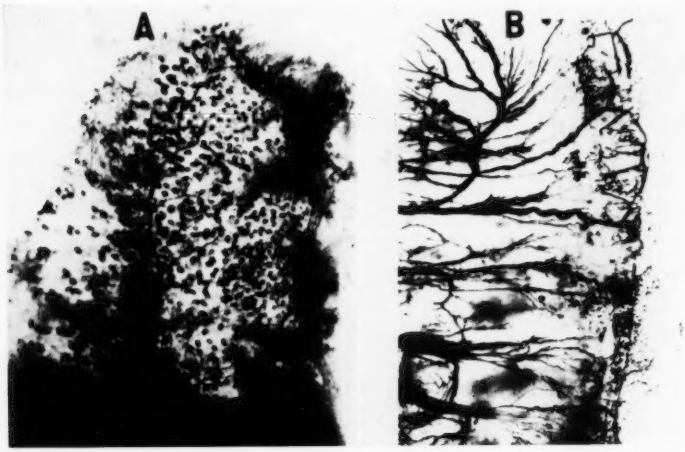


Fig. 5. Photomicrographs (× 8.5) of kidneys injected with Berlin blue via the aorta. A, *R. catesbiana*, ♂, 215 grams, after perfusion with fully oxygenated Ringer. Arterial perfusion flow before excision, 3 cc. per minute; urine rate, 2.8 cc. per hr.

B, *R. catesbiana*, ♂, 100 grams, after perfusion with $O_2$-free Ringer. Arterial perfusion flow before excision, 0.9 cc. per minute; urine rate, less than 0.02 cc. per hour.

Equally convincing evidence can be presented to show that similar difference between the reaction of renal and extra-renal arteries to lack of oxygen exists. In one of the experiments referred to on page 679, in which urine flow and arterial perfusion flow were found to undergo parallel decrease during arterial perfusion with $O_2$-free Ringer, one iliac artery was separately perfused. Flow through it increased to nearly the same extent as that to which the renal perfusion flow decreased.[7] The charted record of this experiment is similar to that shown in figure 4.

[7] Gastro-intestinal vessels, perfused through the coeliaco-mesenteric artery, were dilated both by M/1500 HCN and by $O_2$-free Ringer.

Additional evidence was secured in efforts to obtain injection preparations which should demonstrate the number and distribution of the extra-renal branches of the urogenital arteries. At the end of many of the experiments, a 2 per cent solution of Berlin blue was introduced into the arterial perfusion stream. After a few seconds the kidneys were excised, fixed in 10 per cent formalin, dehydrated with alcohol, and cleared with benzol, benzyl benzoate and methyl salicylate. When the perfusion fluid to which the dye was added was fully oxygenated the color pattern of the kidney was dominated by the glomerular injection (fig. 5A). When however dye was injected at the end of a period of arterial perfusion with $O_2$-free Ringer, the number of stained glomeruli was small and the intensity of staining slight, whereas the branches of the urogenital arteries to the ureters and Mullerian ducts were conspicuously filled (fig. 5B).

Further evidence concerning $O_2$-lack is to be found in the work of Adolph (10), even more impressive in that his experiments were made with living frogs, prepared for direct microscopic observations of the renal circulation. When the animal was subjected to an atmosphere of nitrogen, circulation through the visible glomeruli ceased entirely because of constriction of the true renal arteries. The renal portal circulation remained vigorous.

> The branches of the renal arterioles that supply blood to the mesentery lateral to the kidneys and to the Mullerian ducts were also observed. These arterioles and the larger branches of the renal arteries leading to them could be seen in great detail. . . . In them the flow of blood diminished slightly when asphyxia developed. [Aortic pressure fell during asphyxia by about 40 per cent of its initial value.] Their lumina always remained patent, and the blood usually persisted in flowing through them. . . . The response [constriction] was often limited to the truly renal arterioles, thus not including the separate arterioles that led to the oviduct.

From this it is clearly apparent that the true renal arteries are constricted by asphyxia; the extra-renal arteries are not.

We have not extended these experiments to include study of the vascular actions of phenyl urethane. In our experience the Höber effect with phenyl urethane was too elusive to warrant the effort. It may be mentioned that David (11) found that low concentrations of phenyl urea and isoamylcarbamate, two narcotics in the group with phenyl urethane, constricted the renal vessels but did not constrict those of the legs.

*Other observations.* The permeability of the frog's glomerular membrane is increased by CN. This statement agrees with the observations of Starling and Verney (13) made on the perfused dog's kidney. It is based on 6 experiments with bull frogs, in two of which crystallized horse serum albumin was added to the arterial perfusion fluid. Urine collected during perfusion with M/1500 HCN contained unmistakable traces of albumin; that collected during the control periods before and after HCN, did not. In the four other experiments trypan blue was added to the

arterial perfusion fluid. Its concentration in the urine of the CN period was usually greater than that in the previous control period and in every case more of the blue component of the dye was excreted.

This effect on permeability can be regarded as responsible, at least in part, for the very marked increase in urine which consistently occurred in the majority of our experiments in the post-cyanide periods. The change was so abrupt and so marked as to suggest the sudden release of the urinary mechanism from an inhibiting influence when the CN-Ringer was replaced with normal Ringer. Constriction of the renal arteries may well have acted as such an inhibiting influence.

It is certain also that under the influence of cyanide other vessels in the kidney than the glomerular capillaries become increasingly permeable. In our experience it has never been possible to collect from the post-caval vein more than 95 per cent of the fluid which flowed into the aorta and anterior abdominal vein from the perfusion bottles. Usually, though not invariably, the leakage became greater during CN perfusion. And in experiments in which the two kidneys of a frog were perfused separately via the renal portal veins addition of HCN to make M/1500 in the fluid to one kidney caused that kidney in comparison with its opposite to become noticeably swollen and wet.

*Osmotic effect of CN.* Another possible factor in the action of cyanide on the perfused frog's kidney is the effect which it may exert upon the permeability of tubule cells with resultant swelling due to entrance of water. Increased permeability to water in the presence of HCN was demonstrated by Blumenthal (14) in experiments with Arbacia eggs; we find evidence of its occurrence in kidney tissue. The two kidneys of a large bull frog after brief perfusion via the anterior abdominal vein with oxygenated Ringer's solution were quickly excised, dissected free from connective tissue, blood vessels and ureters, slashed longitudinally with a razor to increase accessible surface, dried with filter paper and weighed separately in stoppered weighing bottles. One was then immersed in hypotonic (1:2), oxygenated Ringer's solution; the other in Ringer of the same concentration containing M/1000 HCN. At intervals they were taken out, dried with filter paper, weighed, and returned to the solution. The following figures show the percentage increases in weight of the two kidneys:

| | 3 MINUTES | 6 MINUTES | 10 MINUTES | 20 MINUTES | 35 MINUTES |
|---|---|---|---|---|---|
| Control | 15.4 | 28.8 | 37.6 | 57.4 | 70.1 |
| HCN | 11.4 | 27.4 | 39.7 | 64.3 | 79.4 |

Two other experiments gave similar results. From the swelling of cells which these results indicate and from the edema of the kidney described

above we may conceive that cyanide may produce a narrowing of the lumina of tubules sufficient to retard the passage of urine through them and perhaps to increase resistance to glomerular filtration.


## SUMMARY

The production of oliguria in the perfused frog's kidney by arterial perfusion with CN or with $O_2$-free Ringer has been ascribed in the past to depression of a secretory mechanism within the glomerulus, because measurements of total arterial perfusion flow were interpreted to mean that glomerular flow and pressure were not altered by these agencies. Evidence presented in this paper shows that arteries which do not supply glomeruli, as well as those which do, are reached by the arterial perfusion fluid. These two types of vessels do not react alike to CN or asphyxia; the former dilate, or do not constrict; the latter usually constrict. As a net result, glomerular perfusion flow and glomerular capillary pressure may decrease with little change in total arterial perfusion flow. The phenomenon of oliguria from CN or asphyxia can therefore be regarded as due to lessened filtration.

Cyanide increases the permeability of the frog's glomerulus. This effect may or may not be exhibited as increase in rate of urine formation, depending upon the degree to which it is antagonized by the constrictor action of this poison on the glomerular arteries and arterioles. The marked increase in urine which usually occurs in post-cyanide perfusion periods is thought to result from this effect.

Exposure to dilute solutions of cyanide increases the permeability of tubule cells to water.


For determinations of Cl and pH we are indebted to Miss E. H. Shiels.

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# THE RESPONSES OF THE SUPERIOR CERVICAL GANGLION TO SINGLE AND REPETITIVE ACTIVATION

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Received for publication January 27, 1938

In a previous study from this Laboratory (Cannon and Rosenblueth, 1937) it was reported that there is a marked increase of the contractions of the nictitating membrane in response to maximal single preganglionic nerve volleys when delivered after a brief period of tetanization. This post-tetanic increment was attributed to repetitive discharges from the ganglion as the most likely explanation. The present study was undertaken in an attempt to record electrically such repetitive discharges in the postganglionic fibers.

The observations made for the purpose of solving that problem soon revealed the desirability of extending the study to include other experimental conditions—mainly the effects of repetitive stimulation at various frequencies, a topic only little studied (cf. Bronk, Tower and Solandt, 1935; Bronk and Pumphrey, 1935). The present report deals mainly with such effects of repetitive stimulation and with their bearing on the theories of synaptic transmission.

METHOD. Thirty-nine cats were used, under dial anesthesia (Ciba, 0.7 to 0.8 cc., intraperitoneally). One or both superior cervical ganglia were exposed and isolated by removal of the X, XI and XII adjacent cranial nerves, sometimes also the IX, care being taken to preserve intact the circulation of the ganglion. That the circulation of the ganglia was adequate was shown by their pink color; gentle stretching or pressure resulted in pallor, which disappeared upon release.

Silver-silver chloride electrodes, shielded by rubber from the surrounding tissues, were applied for recording purposes to the ganglion or to some or all postganglionic branches. The interelectrodal distance was usually from 3 to 5 mm.; exceptionally shorter intervals were employed, when recording from short postganglionic strands. The nerves were sometimes intact; more frequently they were crushed at the level of the electrode placed cephalad. Similar electrodes were used for stimulation of the cervical sympathetic, one or two pairs being placed low in the neck after cutting the nerve centrally.

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Normal moisture and temperature were secured by packing lightly with cotton and covering with the muscles and skin. Although the baseline of the records was occasionally rough, because of the neighboring muscles, and although the stimulus artifacts were sometimes more prominent than they would have been if the nerves had been more isolated, the procedure described was adopted as affording optimum physiological conditions.

Several types of stimuli were delivered to the nerves. For single-shock activation the following stimuli were used: condenser discharges (capacities 0.2 to 0.05$\mu$F); brief pulses from a photoelectric cell obtained by means of a focal plane photographic shutter, amplified to the desired voltage and rendered diphasic by passage through a transformer; make and break induction shocks from a Harvard coil. For repetitive stimulation the photoelectric cell was used; frequencies from 1 to 240 per sec. were obtained from slits in rotating disks driven by a synchronous motor. In some experiments make and break shocks from a Harvard induction coil were timed by a mechanical interrupter.

The electric responses were usually led to a 5-stage capacity-coupled amplifier and photographed from a cathode-ray oscillograph. For a more accurate study of the slow potentials recorded from the ganglia a Hindle string galvanometer was employed. Even without amplification a slack string will reveal the relatively large potentials developed during repetitive activation. A direct-coupled amplifier was, however, ordinarily used to render the records more readily measurable.

Results. I. *Responses to single preganglionic volleys.* The lead off electrodes were placed both on the ganglion, or one on the ganglion and the other on the postganglionic fibers, or both on some or all of the postganglionic branches. For reasons which will appear below the records in which one or both of the electrodes were on the ganglion will be called ganglionic records as opposed to the postganglionic records, obtained when both leads were on these fibers. The grid lead was always placed cephalad. The nerves were either intact, yielding diphasic records, or, more frequently, crushed at the level of the distal grid electrode, yielding approximately monophasic records.

In any of the conditions mentioned a series of single shocks applied to the preganglionic nerve with increasing intensity leads to the appearance of electrograms progressively more complex by the addition of spike potentials, indicating the activation of more or less well-synchronized groups of fibers with different latencies.

The first group to appear is the one with the shortest latency (cf. fig. 1), the M1 fibers of Bishop and Heinbecker (1932). Occasionally this wave is composite, indicating that subgroups are present within it (Bishop and Heinbecker, 1932; Eccles, 1935a). Before the spike from this group has

become maximal the second spike (M2), with a longer latency, begins to grow (cf. fig. 1), thus showing a significant overlap of the thresholds of the corresponding preganglionic fibers in the two groups. As the stimulus is further strengthened new groups of fibers become activated in degrees which vary for different animals.

Frequently, and almost always if the ganglion has been previously maximally tetanized (see section IV below), the next group to appear is revealed by a spike of much longer latency, the U group of Bishop and Heinbecker (fig. 1). This spike may begin to develop even while M2 is still submaximal, and, after a tetanus, when M2 is barely liminal (fig. 12).

With a stronger shock the space in the electrograms between M2 and U usually becomes irregular, showing quite asynchronous nerve impulses which may synchronize in some animals into different recognizable waves.

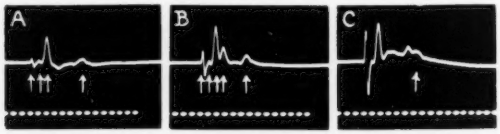


Fig. 1. Postganglionic record. Distance between stimulating and recording electrodes: 5 cm.

A. Response to a weak shock. The arrows indicate successively the stimulus artifact, then the M1, M2 and U spikes.

B. Response to a stronger shock. The arrows indicate the stimulus artifact, then the M1, M2, M3 and U spikes.

C. Response to a still stronger shock. The arrow indicates a spike later than U.

In this and the succeeding electrograms negativity of the caudad ground lead is shown by upward excursions. The cathode-ray oscillograph records appear as white tracings on a dark background, while the string galvanometer records are black on a white background. The short intervals in the time signal of the cathode-ray oscillograph records denote 10 msec.

Of these the most constant occurs during the subsiding phase of M2. This is the M3 group (fig. 1); in some animals, however, other waves may become prominent in this region of the electrogram, thus yielding very complex patterns. This complexity may be further increased by the not uncommon appearance of a wave even later than U (fig. 1C).

The ganglionic records differ from the postganglionic in that the spikes from the ganglion overlap more than they do from the postganglionic fibers. The spikes of the M1 and M2 groups led from the ganglion are sometimes separated by only a minor break in the ascending phase of the first spike. When maximal, M2 is invariably greater than M1 or any of the later spikes. In some animals, before and even after a tetanus, M3 may have a lower threshold than U.

The ganglionic electrograms show other changes of potential than

the spikes just described. The spikes of the M1 fibers, and especially those of the M2 group, are succeeded first by a negative after-potential (the N wave of Eccles, 1935c) and later by a prominent and prolonged positive after-potential (the P wave of Eccles).

The negative after-potential becomes apparent as soon as the spikes recede. Its magnitude and duration are difficult to determine because of the superimposed late spikes. About 50 msec. after application of the stimulus, without any abrupt change the ganglion ceases to be negative and begins to be positive with respect to the postganglionic axons. The positive after-potential attains its peak of about 100 to 200$\mu$ V., approximately 120 msec. after stimulation. It then slowly declines and disappears about 400 msec. later.

Whether the recording be monophasic or diphasic does not alter significantly the positive after-potential, either in magnitude or in duration. The negative after-potential may become obscured by the second phase of the spikes in the "diphasic" leads from uncrushed ganglion and nerves; but the positive after-potential shows no tendency to become diphasic in such conditions.

The differences between the postganglionic and the ganglionic electrograms can best be observed as follows. A pair of recording electrodes is first applied to the postganglionic fibers, crushed at the level of the cephalad electrode, and then moved progressively toward the ganglion with a new crush corresponding to each step. The records from the ganglion show then an increase in the magnitude and duration of the spikes, and an even more marked increase in the magnitude of the after-potentials, as compared with the postganglionic records (fig. 2). The responses obtained with one lead on the ganglion and the other on the postganglionic fibers closely resemble those recorded with both leads on the ganglion.

II. *Responses to two volleys.* The purpose of these observations was to examine the influence of a first (conditioning) nerve volley on the responses to a second (test) volley delivered at variable intervals after the first one. Such observations have been extensively made by Eccles (1935b and c). The present results were similar whether the responses were recorded from the ganglion or the postganglionic fibers.

Three methods were employed. The first two responses in a tetanic series (see section III) were examined and could reveal changes in the second one. This method detected changes in the response to a second of two identical volleys, maximal or submaximal. In some cases a maximal or submaximal volley was interposed at random during a series of maximal or submaximal responses at a slow, continuous frequency (0.5 to 2 per sec.). Finally, the results of applying two break induction shocks at variable intervals, timed by a Lucas pendulum, were also recorded.

Submaximal shocks, in our experience, do not yield constant responses, even when applied at long intervals. Such variability is not surprising in view of Blair and Erlanger's (1933) report of spontaneous changes in the threshold of nerve fibers; such changes might be quite significant when sampling a large population of fibers with approximately similar thresholds. Because of this variability the magnitude of the spikes of the test volley shows considerable scatter when plotted against the interval between the two volleys (cf. fig. 5). The degree of scatter was judged an adequate criterion for the validity of the effects encountered.

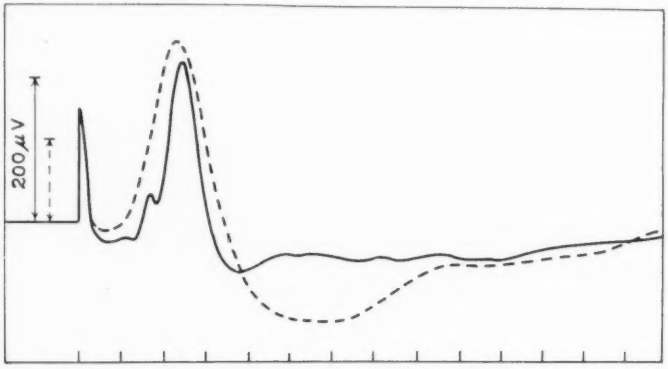


Fig. 2. Differences between postganglionic and ganglionic responses to single maximal preganglionic volleys. The recording electrodes were placed first (solid line) with the ground just beyond the tip of the ganglion and the grid 4 mm. cephalad (nerves crushed), then (broken line) they were moved caudad till the grid lead was at the tip of the ganglion (crushed). The amplification was greater for the postganglionic than for the ganglionic record, as shown by the calibrations preceding the stimulus artifact. Time: 10 msec. intervals. The duration of the positive after-potential of the ganglionic response was considerably curtailed by the condenser-coupled amplifier. Traced from the original records projected by a photographic enlarger.

The changes in the magnitude of the test spikes as a function of the interval between the two volleys varied considerably from one animal to another; they varied also when different methods for testing were employed on the same animal (e.g., the intercalated random volley vs. the two-break-shock method); they finally also varied markedly with a single method and in a single animal for the M1 and the M2 spikes, respectively.

Within this variability the following results were generally obtained (cf. fig. 5). A test volley applied immediately after a maximal conditioning volley elicits no response because of the refractoriness of the cells. With

intervals of 2 to 3 msec. a response begins to appear which increases progressively, to attain a normal magnitude usually at intervals of about 10 to 20 msec. for M1 and 15 to 30 msec. for M2. This progressive growth is probably due to recovery of the cells from refractoriness. The responses, both M1 and M2, may continue to grow at longer intervals, reaching a maximum at about 20 to 100 msec. intervals. The M1 responses usually decline thereafter from this maximum to become normal 100 to 300 msec. later. The M2 responses may behave similarly (cf. fig. 2, Rosenblueth and Simeone, 1938), or may occasionally show a decrease at longer intervals (100 to 400 msec.) and as a rule are normal after 600 msec. Exceptionally, however, a marked increase of both M1 and M2 may be detected with test volleys applied up to 1 sec. after a conditioning volley (cf. fig. 3).

As will appear in the discussion, it is important to emphasize that whether the test volley is maximal or submaximal does not change the results—i.e., large increases (up to 35 per cent) of the ganglionic responses,

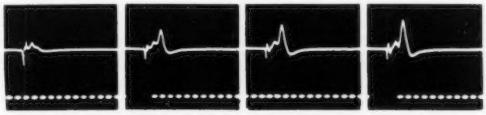

Fig. 3. Progressive increase of the responses to successive shocks during repetitive stimulation. Submaximal volleys delivered at intervals of 500 msec.

both M1 and M2, to maximal volleys may be observed when applied at a suitable interval after a conditioning volley.

The above description of the influence of a conditioning volley on the responses to a test volley disagrees with Eccles' (1935b and c) summary of his observations. Eccles describes a "facilitation" of submaximal test volleys with a peak at about 25 msec. and "inhibition" (depression) with longer intervals (peak at about 100 to 150 msec.). This "facilitation" and "inhibition" he correlates with the negative and positive after-potentials of the conditioning volley, respectively. Such a correlation will be considered critically later. It may be pointed out, however, that "facilitation" occurred in his, as in the present experiments, mainly for M1 spikes, "inhibition" mainly for M2. Although Eccles adopts a uniform description of his results, considerable variability is obvious in his published records (compare figs. 3, 11 and 12 of his 1935c paper). An increase of of maximal test responses was also observed by Eccles (1935a, p. 199). He attributes such an increase to a synchronization of the corresponding elements since he considers the area as "probably unaltered." This explanation does not apply to the present results; the increase of magnitude was usually associated with an increase of area (fig. 4).

The responses to test volleys may be modified by the conditioning volley not only in their magnitude, but also in other respects: latency, synchronism and after-potentials. These changes are more prominent upon repetitive stimulation. They will be described below.

III. *Responses to repetitive stimulation.* The responses to successive shocks during repetitive stimulation show striking changes in the latency, magnitude and pattern of the spikes and in the magnitude and duration of the after-potentials.

Slow frequencies of stimulation of the ganglion (up to 18 per sec. for about 5 sec.) do not lead to any significant change in the latency of M1. Higher frequencies (e.g., 30 to 60 per sec.) produce frequently an initial decrease of latency (cf. Eccles, 1935a), so that the response to the 10th shock may occur as much as 1 msec. earlier than at the start. This decrease is succeeded by an increase which occurs quite rapidly during the first two seconds of stimulation and proceeds thereafter more slowly

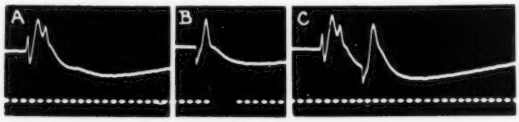


Fig. 4. Increase of the response to a test volley when occurring during the positive after-potential of a conditioning volley. Both stimuli delivered through the same electrodes to the preganglionic nerve trunk.

A. Conditioning volley alone.

B. Test volley alone.

C. Test volley applied 52 msec. after the conditioning volley.

(fig. 6). After 10 sec. of repetitive activation the latency of M1 may be as much as 2 msec. longer than at the beginning.

Similar changes of latency occur for the other spikes of the electrograms, with quantitative differences which may be summarized as follows. A given moderately fast frequency will induce a greater increase of latency for the late than for the early spik (cf. M1 and M2 in fig. 6); at 30 per sec. the latency of U may increase as much as 7 msec. after 2 sec. of stimulation. The late spikes begin to show increased latencies after a given period of stimulation with lower frequencies than those which affect the early spikes; thus, 18 per sec. for 5 sec. will significantly increase the latency of U while that of M1 will be practically unchanged; thus, also, during the first 0.1 sec. of stimulation at 60 per sec. the latency of M1 may decrease while that of M2 may increase. For any of the spikes, the greater the frequency and the longer the period of stimulation, the greater the increase of latency obtained at the end.

The initial decrease of latency during repetitive stimulation seems to

have an optimum frequency for its appearance. For M1 this optimum is at about 40 per sec. Frequencies faster than about 60 per sec. always produce an increase of latency from the start.

The increases of latency after some time of frequent stimulation are greater for the peaks of the spikes than for their beginning, thus showing that the temporal dispersion of the fibers in each of the groups is increased (Bronk and Pumphrey, 1935). This increased temporal dispersion is also shown by the greater duration of each of the waves. The group M3

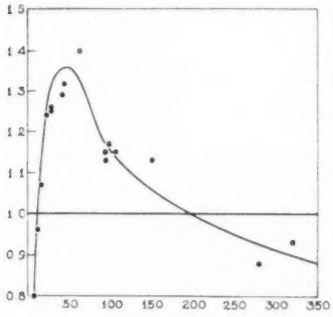


Fig. 5

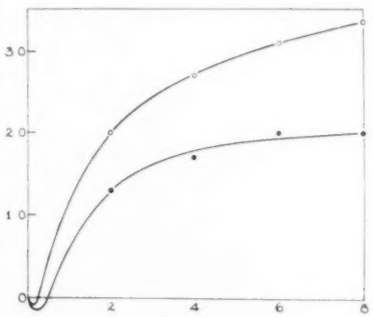


Fig. 6

Fig. 5. Changes in the magnitude of the M2 spike of a maximal test volley applied at variable intervals after a maximal conditioning volley. One pair of stimulating electrodes; Lucas pendulum. Ordinates: ratio of the conditioned responses to the response to the test volley alone. These conditioned responses were measured by subtracting from the record of the response to the two volleys the response which the conditioning volley produces alone (for a discussion of this method see Rosenblueth and Simeone, 1938). Abscissae: interval between the two volleys in milliseconds.

Fig. 6. Changes of latency during repetitive stimulation at the rate of 30 per sec. The latency of M1 was measured at the beginning of this spike; that of M2, at its separation from M1. Ordinates: changes in lateney in milliseconds. Abscissae: time in seconds after the beginning of stimulation. Dots: M1; circles: M2. The latency of M1 for the first shock was 7.4 msec.; that of M2, 15.8 msec.

shows this dispersion more than any other, more even than U, which appears to be more resistant from this standpoint.

The changes of magnitude of the spikes during repetitive stimulation were studied mainly for the M1 and M2 groups. Such changes may consist in either a growth or a decrease of the spikes. The results were similar for the ganglionic and the postganglionic responses.

Whether the stimulating shock be submaximal or supramaximal for M1 and M2 the two spikes usually grow after a few seconds (from 2 to 5) of repetitive activation with slow (2 to 18 per sec.) frequencies of stimulation (cf. Bronk, Tower and Solandt, 1935). This growth was sometimes

more marked with submaximal than with maximal shocks. The phenomenon is especially striking when, beginning with a barely liminal response, good-sized spikes occur after a few seconds of stimulation (fig. 3).

When the frequency of stimulation was about 30 per sec. the changes in size of M2 after 2 sec. were inconsistent and not marked; M1 usually grew slightly with this frequency. Higher frequencies (60 to 120 per sec.) led to a small decrease of M1 and a larger decrease of M2 (cf. Bronk and Pumphrey, 1935).

If the periods of stimulation are more prolonged (e.g., 10 sec.) the initial increase for frequencies from 18 to 30 per sec. is followed by a progressive decrease. This decrease is greater for M2 than for M1 and greater for the higher than for the lower frequencies. Thus, at the end of 10 sec. of stimulation at 30 per sec. M2 may be less than one-half of its original magnitude (cf. fig. 8).

There is frequently a discrepancy between the effects of the first two shocks and those of later ones, i.e., the response to the second shock may

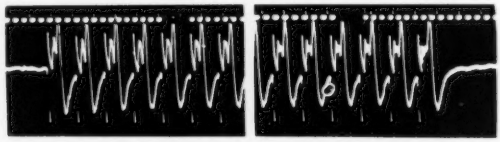

Fig. 7. Repetitive discharges of M1 cells during tetanus. Beginning and end of maximal stimulation at the rate of 30 per sec. for 1.3 sec. Diphasic recording from the uncrushed postganglionic fibers.

be smaller than that to the first while later shocks elicit markedly increased spikes (cf. fig. 7, Rosenblueth and Simeone, 1938). Repetitive stimulation may lead to the appearance of extra M1 and M2 waves which probably denote repetitive discharges of some of the ganglion neurones to some of the nerve volleys delivered during the tetanus. Such evidence for repetitive discharges was present clearly only in some of the animals studied, and only after short periods of stimulation with moderate frequencies (e.g., 30 per sec.). Figure 7 illustrates the phenomenon; it will be noted that the appearance of new M1 waves is not attended by a significant decrease of the original M1 spike, which was maximal from the start.

Some data concerning the ability of the different fiber groups, corresponding to the different spikes, to follow repetitive activation at increasing frequencies have already been mentioned in relation to the changes in magnitude during tetani. Judged by that and other criteria—e.g., amount of temporal dispersion and alternation—the following statements can be made. Up to a frequency of 18 per sec. all four groups of fibers follow the stimuli for 2 to 5 sec., without any evidence of failure of any

of the corresponding cells. At a frequency of 30 per sec. M3 drops out entirely from the records as a recognizable wave after a few shocks, probably because of great temporal dispersion; U is still clearly defined after 2 or 3 sec. stimulation (fig. 8). At 60 per sec. U disappears after a few shocks. It may be concluded, therefore, that the order of the groups as regards their ability to follow high frequencies of stimulation is the following: M1 > M2 > U > M3.

Different ganglia may behave quite differently with respect to their after-potentials upon repetitive stimulation. Notwithstanding these differences, to be described below, certain uniformities were encountered. With slow frequencies of stimulation (below 20 per sec.) the ganglion is positive between the spikes throughout the period of stimulation—i.e., each spike starts during the positive after-potential of the preceding

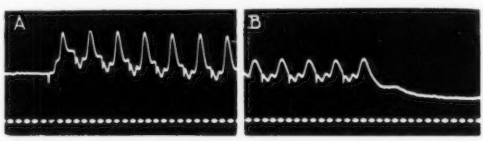


Fig. 8. Postganglionic responses to maximal repetitive stimulation at the rate of 30 per sec.

A. Beginning of stimulation. The stimulus artifact of the first shock is succeeded by clearly separated M1, M2 and M3 spikes. The second stimulus artifact occurs during the first M3 spike. M3 rapidly disappears as a recognizable wave while M2 grows in magnitude.

B. End of stimulation (5 sec. later). The last shock elicits smaller M1 and M2 spikes than in A. Increased latencies and temporal dispersion are apparent. A U wave is obvious although M3 is not detectable

responses (figs. 9 and 10). This positivity is sometimes greater than that which a single shock develops—i.e., the positive after-potentials of succeeding shocks may sum; but sometimes the ganglion never becomes more positive than a single discharge will render it. With high frequencies (above 25 per sec.) the ganglion is negative throughout stimulation, the more negative the higher the frequency; indeed, this negativity may attain values greater than those of the first spikes (fig. 10).

At the end of stimulation, if the ganglion was positive (slow frequencies), it will return slowly to base-line; if the ganglion was negative (fast frequencies) it will either return slowly to base-line, or, more commonly, it will cross the base-line and enter a prolonged period of positive after-potential. The differences in behavior for different ganglia lie mainly in their relative tendency to show large negative and small positive after-potentials (fig. 10) or vice-versa (fig. 9).

For any given ganglion the magnitude and duration of the positive

after-potentials increase with the duration of stimulation at a constant frequency, up to 15 sec.—no longer durations were tested. The same direct

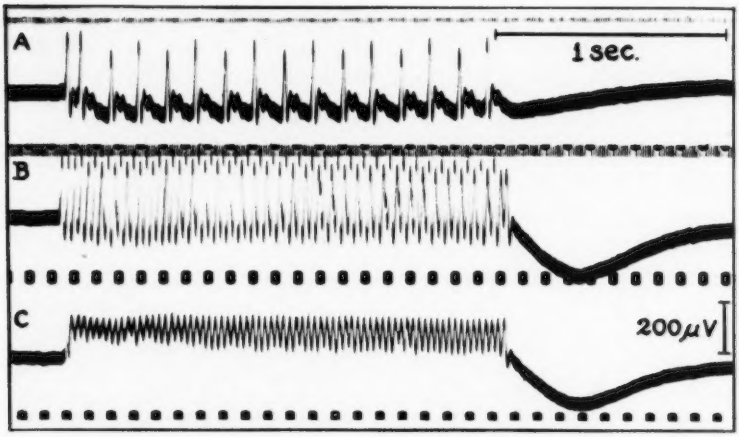


Fig. 9. Summation of after-potentials during tetanic stimulation. Make and break induction shocks as stimuli.
A. Frequency: 4 pairs per sec.
B. Frequency: 18 pairs per sec.
C. Frequency: 70 pairs per sec.

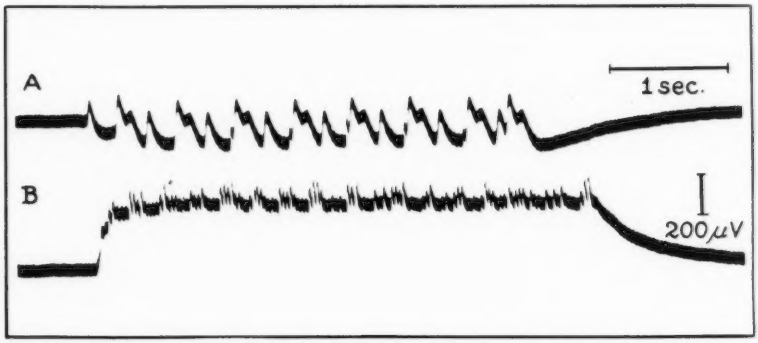


Fig. 10. Summation of after-potentials during tetanic stimulation. Make and break induction shocks as stimuli.
A. Frequency: 2 pairs per sec.
B. Frequency: 60 pairs per sec.

relationship was found for the duration of the tetanus and the magnitude and duration of the negative after-potential.

The greater the frequency of stimulation for a given period, the greater

and the longer the negative after-potential. In one experiment stimulation for 15 sec. with a frequency of 130 per sec. yielded 400$\mu$V negativity, which subsided only after 2 sec. As regards the relations between frequency of stimulation and magnitude of the positive after-potential there is usually an optimum frequency, different for different ganglia, beyond which the increasing negativity lessens the later positivity. The maximum positivity recorded was 400$\mu$V and the maximum duration was at least 5 sec.

IV. *Post-tetanic changes.* Under this heading will be described transient changes in the responses to single nerve volleys which occur after cessation of a brief (1 to 30 sec.) repetitive (18 to 90 per sec.) period of stimulation. These changes affect, as in the preceding section, the latency, magnitude and pattern of the spikes, and the magnitude and duration of the after-potentials.

During tetanic stimulation at moderate frequency (e.g., 30 per sec.) the latency of M1 and M2 first decreases, then increases (section III). Single shocks applied shortly (0.2 to 1 sec.) after a period of tetanic stimulation elicit spikes with latency shorter or longer than normal, depending upon the degree of tetanization. For example, if a prolonged repetitive stimulation at 30 to 60 per sec. is applied, the latency of M1 and M2 may be markedly lengthened for the last shock in the series. A single volley 0.5 sec. later will elicit responses still with increased latency. Successive single shocks applied every second will provoke responses with latencies which first decrease rapidly, and then more slowly, until 1 to 5 minutes later the normal latencies are recovered. The rapidity of recovery depends upon the frequency and duration of the tetanic stimulus; if the frequency is high and the duration long, recovery will be slower than for slower or briefer tetani.

The changes of latency of responses to single shocks after a tetanus may occasionally differ from those described above. For example, in one animal the latency of M2 to the last shock of a period of 3.5 sec. of repetitive stimulation at the rate of 80 per sec. was 18 msec.; the latency to a single shock 1 sec. later was 13.5 msec.; 3 sec. later it was 12.5 msec.; 3 sec. later it was 13.5 again; and 2 minutes later it had returned to the normal value of 13.8 msec.

In general, after a given long period of fast repetitive stimulation the increase of latency is more marked and the recovery is more delayed for the slower than for the faster fiber groups. Thus, in one instance, the latency of U after a tetanus was increased by 10 msec. and recovery did not occur till about 5 minutes later.

The usual effect of tetanic stimulation upon the magnitude of M1 and M2 to subsequent single shocks is a marked increase (fig. 11) followed by a slow (several minutes) return to normal. If the tetanic stimulus

is rapid and prolonged, immediately after the tetanus the spikes are smaller than normal; they then rapidly grow beyond normal and slowly subside as before. The post-tetanic increment was quite as striking with maximal as with submaximal test volleys; it was also fully as marked in the postganglionic as in the ganglionic records.

It is difficult to decide whether the duration of the spikes is changed during the post-tetanic period, because the large negative after-potential (see below) usually obscures the end of the spikes. Judged by selected

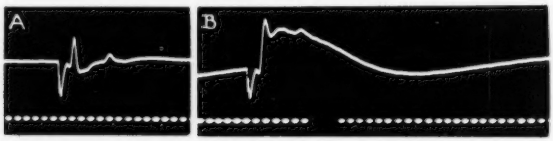


Fig. 11. Post-tetanic increment of M2 and of the negative after-potential. Postganglionic record. Maximal condenser discharges, A, before, and B, 300 msec. after repetitive (30 per sec.) stimulation for 2.5 sec. The M1 spike begins before the stimulus artifact has subsided; M3 is not well defined; the late spike is U. The latency of M2 in A is 12.2 msec.; in B, 13.1 msec. The latency of U in A is 50.5 msec.; in B, 52.2 msec

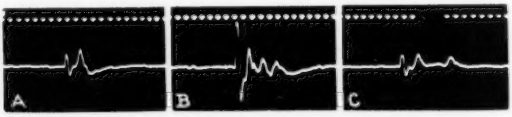


Fig. 12. Effect of a previous tetanus upon the threshold of the U group. Postganglionic record.

A. Weak shock before tetanizing. The M1 spike occurs during the stimulus artifact. A submaximal M2 is apparent. No U wave is present.

B. Strong shock before tetanus. M2 becomes maximal and is succeeded by 2 M3 waves (small and large) and finally by a U spike with a latency of 44 msec.

C. Weak shock (same intensity as in A) 15 sec. after tetanizing with an induction coil for 30 sec. A U spike now occurs with a latency of 56 msec.

favorable instances it is probable that the duration of the M2 spike is increased after a tetanus.

The U neurones are peculiar as regards their behavior during the post-tetanic period. Very slight increases, if any, of the U spikes to maximal shocks were observed. On the other hand, weak shocks, which elicit only M1 and submaximal M2 spikes before a tetanus, usually evoke after a tetanus a full-sized U spike, although M2 remains submaximal. The latency of this U spike is prolonged, as is customary after a tetanus (see p. 699). Figure 12 illustrates a typical instance.

The negative after-potential in response to a single volley is greatly increased after a tetanus (fig. 11). This increment appears immediately

after the period of tetanic stimulation, even while the ganglion is still in the phase of post-tetanic negative after-potential, and may outlast the tetanic positive after-potential. The positive after-potential to single post-tetanic shocks is, on the other hand, usually much decreased immediately after the tetanus and remains small while the tetanic positive after-potential lasts. Later (30 to 60 sec.) single shocks may elicit larger positive after-potentials than before the tetanus.

When the records are taken from the postganglionic fibers, large negative after-potentials to single shocks may occur after a tetanus much as in the ganglionic records (fig. 11). These after-potentials are especially striking since the normal postganglionic records show only very small after-potentials (section I). It is possible that they may spread from the ganglion to the postganglionic fibers (cf. Eccles, 1935a).

Discussion. A. *The fiber groups.* The results presented in section I differ from those reported by Eccles (1935a) in only one significant respect. While Eccles found that as a rule the threshold for the U spike was higher than that for the M3 group, in the present observations U appeared usually with shocks which were subthreshold for M3 (fig. 1). The present data (sections I and IV) lead to the conclusion that the neurones responsible for the U spike may be supplied not only by the U group of preganglionic fibers, but also by the M1 and the M2 groups. This conclusion accounts readily for all the results available. In some cases the M1 and M2 preganglionic impulses would be subliminal for the U neurones, whereas in other experimental conditions, particularly during the period of post-tetanic high excitability (fig. 12), such M1 and M2 impulses would be sufficient for activation of the U elements.

The U group is further remarkable for its great resistance to repetitive stimulation, greater than that of the faster group M3 (fig. 8). This resistance depends in all probability on the properties of the synapses, not of the axons, for the axons, even the slow ones in the postganglionic fibers, should be able adequately to follow higher frequencies than those at which a failure appears when transmitted through the ganglion (Bronk and Pumphrey, 1935). The synaptic delay is greatest for the U fibers (cf. Eccles, 1935a; the present results agree with those he reported). It thus seems likely that synaptic delay and ability to follow high frequencies of stimulation are independent variables.

B. *The source of the ganglionic potentials.* Bishop (1936) has pointed out some of the difficulties to be considered when interpreting electrograms obtained from a tapering mass of tissue such as the superior cervical ganglion. The evidence available (cf. Eccles, 1937a), however, provides a strong basis for the conclusion that the potentials recorded from the ganglion arise from elements therein which are not nerve axons—i.e., they arise from either the dendrites, or, more probably, the cell bodies, or both.

It was reported in section I (fig. 2) that the spikes become larger and last longer when the recording electrodes are moved from the postganglionic fibers to the ganglion. If the cell bodies in the ganglion did not contribute to these spikes the inert tissue would act as a shunt; the potentials should then decrease in magnitude, but they do not. Temporal dispersion of the postganglionic impulses should become more prominent in records obtained further away from the ganglion than in those led off at their start; the increased duration of the ganglionic spikes remains therefore unexplained, unless it is accepted that these spikes of greater duration arise from elements which are not the postganglionic axons.

A similar argument applies to the after-potentials. Their great increase in magnitude when the recording electrodes are moved from the postganglionic nerves to the ganglion (fig. 2) pleads for an origin at the ganglion, and the fact that records obtained from intact nerves are practically identical with those recorded after crushing at the level of the cephalad electrode leads to the inference that the after-potentials denote a difference of potential between the cell body and the axon (Eccles, 1935a).

C. *The changes in magnitude of the spikes.* Eccles (1935b and c, 1936, 1937b) in all his papers on the ganglion interprets an increase in the magnitude of a submaximal spike as denoting invariably the activation of more elements in the ganglion—i.e., spatial "facilitation"; and a decrease in magnitude as denoting the activation of fewer elements—i.e., spatial "inhibition." On one occasion (1935a), having found an increase of the response to the second of two maximal preganglionic volleys, he states that this increase need not denote that some elements not activated by the first volley responded to the second one, for synchronization of the response could account for the increment, as the total area was "probably unaltered."

In the present observations (figs. 4 and 11, sections II, III and IV) increments of the spikes to maximal preganglionic volleys were found in the same conditions in which submaximal responses were also increased. The increase of the maximal responses could not be due exclusively to synchronization, for not only was the height, but also the total area, significantly greater.

The data available are insufficient to settle the question whether a a maximal preganglionic volley sets up an equally maximal postganglionic discharge—i.e., whether all the elements of the ganglion are stimulated supraliminally in such conditions. Brown's (1934) observations show that such is probably the case for the fibers which supply the nictitating membrane. No relevant evidence has been reported, however, for the other fiber groups. It is well known that the magnitude of the spike potential of C nerve fibers increases when these fibers conduct during their positive after-potential from a previous volley (Gasser, 1937). An

increment of the magnitude of the ganglionic or postganglionic spikes to a maximal preganglionic volley could therefore be at least partly due to an increment of the spike-size per fiber. It might also be due to the activation of additional elements. The probable ability of the ganglion neurones to increase their spike-size was neglected by Eccles; it renders the conclusion of "facilitation" questionable for some of his experiments. In the remainder of this discussion facilitation will be mentioned only when the increase of responses was so great as to render improbable the explanation by means of changes in spike-size per element.

When a decrease in spike magnitude occurs, e.g., at fast frequencies of stimulation (section III), it may be partly explained by temporal dispersion (Bronk and Pumphrey, 1935). A decrease of the spikes can also be due to a failure of synaptic transmission for some of the neurones in the ganglion; that such failure may take place is conclusively shown by the experiments of Orías (1932; cf. Rosenblueth, Davis and Rempel, 1936). Finally, a decrease of the total spike-potential from a group of fibers might be due to a decrease of the magnitude of the spike per fiber.

D. *The significance of the after-potentials.* Eccles (1935c, 1936) has suggested that the negative after-potential is the sign of C.E.S.—i.e., of a condition which lowers the threshold of the cells to the action of preganglionic nerve impulses; and that the positive after-potential is the sign of C.I.S.—i.e., of a condition in which the threshold of the cells to the impinging nerve impulses is raised. Eccles bases these suggestions on the conclusion that "facilitation" of the effects of submaximal volleys occurs when these volleys reach the ganglion during the negative after-potential of a previous discharge, and that "inhibition" takes place during the positive after-potential of a conditioning volley.

Both Eccles' data and his hypothesis are open to criticism, as follows. Some factors other than changes in the number of active elements, that may affect the magnitude of the responses, were discussed in the previous section. Eccles failed to consider these factors in the interpretation of his results. A further source of error neglected in some of his experiments is the influence of the after-potentials of the preganglionic fibers, and the corresponding changes in the electrical excitability and possibly also in the spike-size of these fibers, on the magnitude of the preganglionic test volleys. Thus, if a given submaximal test stimulus is delivered during the period of positive after-potential of the preganglionic fibers it will activate fewer preganglionic fibers than when delivered to the resting nerves. A decrease in the ganglionic or postganglionic response might then be due exclusively to a decrease of the preganglionic test volley, not to any change in the ganglion.

Even if these difficulties are considered negligible, Eccles' data do not justify his conclusions. It was already pointed out (p. 693) that his

results were not uniform. Such inconsistency is incompatible with the correlations he suggests, for the negative and positive after-potentials in response to single volleys consistently follow time-courses which vary only slightly from one animal to another. In Eccles', and in our own experience, growth of the responses to the second of two volleys is mainly observable for the M1 spike, while a decrease of such responses is usually limited to the M2 group. This difference between the two groups is not readily reconciled with the hypothesis under discussion, for both M1 and M2 cells are assumed to have a sequence of qualitatively similar after-potentials—negative, then positive (Eccles, 1935b).

The present observations (sections II, III and IV) offer many instances in which the spikes vary precisely in the opposite direction to that which Eccles' inferences would predict. Thus, upon repetitive stimulation, maximal or submaximal, increased spikes were usually observed when the frequencies were such that each response took place during the positive after-potential of the preceding response, even at its peak (p. 695). Still more emphatic were the post-tetanic increments, which were maximal at the time when the ganglion was maximally positive, after summation of positivity during the tetanus. Conversely, significant decreases of the spikes may take place at the peak of the negative after-potential (e.g., immediately after a prolonged, rapid tetanus (p. 700).

Other studies of autonomic ganglia, when examined critically, show a similar lack of correlation between the after-potentials and the processes of facilitation and depression. Thus, Obrador and Odoriz (1936) reproduced records of facilitation in the test response of a lumbar sympathetic ganglion when it succeeded the conditioning volley by 80 msec.—i.e., at the peak of the positive after-potential to the conditioning volley. Whitteridge (1937) found inhibition of test responses of the ciliary ganglion at intervals up to 250–300 msec., although the positive after-potential lasts only for 125-150 msec. in this ganglion. Finally, Lloyd (1937) found a large positive after-potential of the inferior mesenteric ganglion but did not report any correlated inhibition.

It may be concluded, therefore, that "facilitation" and "inhibition," when present in the ganglion, may be quite independent of the negative or positive after-potentials. This conclusion is of importance in relation to the problem of the influence of after-potentials upon excitation and inhibition in the central nervous system (cf. Gasser, 1937). Nerves have a supernormal electrical excitability during the negative after-potential (Gasser and Erlanger, 1930) and a subnormal excitability during the positive after-potential (Graham and Gasser, 1934). The suggestion arises that neurones might similarly become supernormal and subnormal in excitability during the corresponding phases.

If impinging nerve impulses acted as electrical stimuli supernormality

of the neurones should lead to facilitation. Lorente de Nó and Graham (1936) have reported, however, that motoneurones may be supernormal to electric stimuli, while they are less responsive than normally when tested by nerve volleys.

Hughes and Gasser (1934; cf. also Hughes, McCouch and Stewart, 1937) found a satisfactory correlation between inhibition of a flexor reflex and the positive potential of the spinal cord. In the ganglia, however, as shown above, such a correlation is exceptional. It may be concluded that, unless the two systems differ fundamentally, other factors than the after-potentials may determine the magnitude of conditioned responses of neurones.

If nerve impulses do not act at synapses as electrical stimuli but excite the succeeding neurones by some other mechanism, then it need not be expected that the two after-potential phases should be attended by increased or decreased responsiveness.

E. *The changes in latency.* All the changes in latency recorded (sections II, III and IV) were probably due to changes in the synaptic delay, for changes in conduction velocity at the frequencies of stimulation used, and with the short conduction distances employed, should lead only to negligible variations of the latent period. The conclusion, therefore, emerges that synaptic delay is susceptible to considerable changes. Thus, Eccles (1935a; the present observations agree with his figures) found that the synaptic delay for a single preganglionic volley is about 3 msec. for M1 and 6.5 msec. for M2. The initial shortening of the synaptic delay during tetanic stimulation may decrease by as much as 2.5 msec. for M1 and 3.5 msec. for M2 (cf. Rosenblueth and Simeone, 1938). The lengthening of the delay after prolonged tetanic stimulation may be as great as 2 msec. for M1 and 6 msec. for M2. The synaptic delay may therefore be reduced to a fraction of a millisecond or increased to twice its usual value.

Eccles and Sherrington (1931) and Eccles (1935b) concluded from their observations on the flexor reflex and on the ganglion that a shortening of synaptic delay is correlated with the process of facilitation. The present data show that such a correlation need not be present. Thus, during the post-tetanic period, large, facilitated responses may be seen with a markedly prolonged synaptic delay (section III, fig. 12).

That synaptic delay is independent of the after-potentials is indicated by the observations made during or after tetanic stimulation (sections II and III). Decreased or increased synaptic delays may occur during the negative after-potential as well as during the positive after-potential.

F. *Synaptic transmission.* The data present serious difficulties to the electric theory of synaptic transmission, according to which the action-potentials of the preganglionic fibers act as electrical stimuli to the ganglion neurones. Without further elaborate assumptions the theory does

not account for the following facts: (1) the lack of correlation between the after-potentials and the responsiveness of the ganglion cells (p. 704); (2) the long synaptic delays of some of the cells; (3) the lack of correlation between synaptic delay and after-potentials (section E); (4) the lack of correlation between synaptic delay and facilitation or inhibition (section E); (5) the repetitive responses to single nerve volleys sometimes encountered (sections II and III).

The Kibjakow-Feldberg theory of transmission by acetylcholine, on the other hand, may explain some of these data as follows. According to this theory synaptic delay should be a function of the rate of liberation of acetylcholine and of the threshold of the cells to the mediator, two factors which may vary independently. A prolonged delay with increased responsiveness is thus readily conceivable, or vice versa. An accumulation of acetylcholine or a decreased threshold of the cells in certain experimental conditions might lead to repetitive responses.

It may not be decided at present, however, whether the chemical theory of synaptic transmission will account for all the data reported. For example, it is not known whether or not the threshold of the ganglion cells to acetylcholine varies during the after-potentials, or during the post-tetanic period. Such knowledge may provide a test for the theory.

G. *The similarity of the ganglionic synapse and the neuromuscular junction.* Two more features in which the ganglion resembles the neuromuscular junction are added by the present data to those previously recognized (Elliott, 1907; cf. Cannon and Rosenblueth, 1937). Like the ganglion (section III), skeletal muscle shows an initial shortening and a later lengthening of the synaptic delay during tetanic stimulation and also shows repetitive discharges to single nerve volleys during a tetanus (Rosenblueth and Morison, 1937). Thus all the data available reveal the fundamental similarity of the transmission process at the two junctions.

## SUMMARY

The electric responses of the superior cervical ganglion and the post-ganglionic fibers to preganglionic nerve volleys were studied in cats. Single shocks, two shocks at variable intervals and repetitive stimulation at various frequencies were used.

At least four groups of elements may be recognized in the ganglion, distinguished by their latency and by the threshold of the preganglionic fibers which activate them (Bishop and Heinbecker, 1932). The slowest group U appears sometimes with stimuli which fail to excite the faster group M3 (figs. 1 and 12).

The ganglionic spike potentials (see p. 701) are succeeded first by a negative and later by a positive after-potential (fig. 2; Eccles, 1935a). These after-potentials sum during repetitive stimulation at adequate frequencies (figs. 9 and 10).

Growth or decrease of the magnitude of the spikes which occurs in different experimental conditions (figs. 3, 4, 5, 8 and 11) is not related to the after-potentials (p. 704).

Increase or decrease of latency, particularly striking during tetanic stimulation (fig. 6) and immediately after (p. 699), is independent of the after-potentials and of the changes of magnitude of the spikes (p. 705).

Repetitive discharges of ganglion cells to single preganglionic volleys may occur during tetanic stimulation (fig. 7) or during the post-tetanic period (p. 700).

The bearing of the data on theories of synaptic transmission is discussed (p. 705).

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# THE ACTION OF ESERINE OR PROSTIGMIN ON THE SUPERIOR CERVICAL GANGLION

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Received for publication January 27, 1938

The Kibjakow-Feldberg theory of chemical transmission of the preganglionic nerve impulses at the superior cervical ganglion attributes this transmission to acetylcholine. Since eserine or prostigmin protects acetylcholine from prompt destruction by cholinesterase it would be expected that these drugs should significantly modify the transmission at the ganglion. Indeed, because Eccles (1935b, 1937) failed to detect any action of eserine on the "facilitation curve" of a submaximal test volley applied at variable intervals after a first conditioning volley, he argued that acetylcholine could not be the mediator of the nerve impulses.

Although several recent publications have shown a marked influence of eserine or prostigmin on the ganglion (Feldberg and Vartiainen, 1934; Brown and Feldberg, 1936; Cannon and Rosenblueth, 1937), the results reported by Eccles challenge explanation. From previous experience we deemed it likely that his negative observations were due to the use of inadequately small doses of eserine. The present report deals with the action of sufficient doses of eserine and prostigmin on the ganglion.

METHOD. Twenty-seven cats were used, under dial anesthesia (Ciba, 0.7 to 0.8 cc. intraperitoneally). The procedures followed for recording the electric responses of the ganglion or postganglionic fibers have been described in the preceding communication (Rosenblueth and Simeone, 1938). The stimulating circuits are also described therein.

For stimulation of the two branches of the annulus of Vieussens artificial respiration was administered; the chest was opened at the first costal interspace, and shielded silver wire electrodes were placed on the nerves after severing all the connections of the stellate ganglion with the central nervous system.

Eserine (4 to 8 mgm. per kgm.) or prostigmin (Roche, 0.3 to 3 mgm. per kilogram) was injected intravenously after a previous injection of atropine (1 mgm. per kilogram). Although this dose of atropine has a negligible influence on the ganglion, the control observations before eserine were made after atropine.

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RESULTS. I. *Responses to single shocks.* The postganglionic responses to single volleys, maximal or submaximal, are not significantly altered in magnitude or duration after injections of eserine or prostigmin. Exceptionally the spikes decrease slightly immediately after administration of large doses of the drugs, but the effect disappears within a few minutes. The latency of the spikes, on the other hand, is as a rule slightly (0.5 to 2 msec.) increased after eserine (3 to 6 mgm. per kilogram). This increase

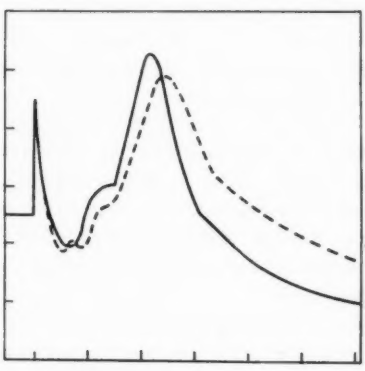

Fig. 1

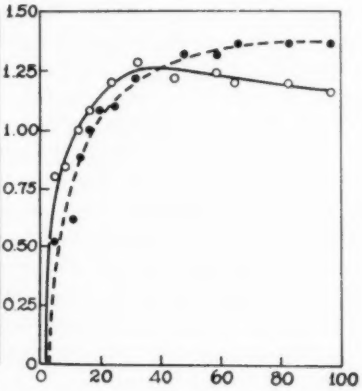


Fig. 2

Fig. 1. Responses of the superior cervical ganglion to a maximal single nerve volley from one of the branches of the annulus of Vieussens. The stimulus artifact is succeeded by the M1 and M2 spikes, which overlap. Full line: before eserine. Broken line: after eserine, 6.1 mgm. per kilogram. Time: 10 msec. intervals. An upward excursion denotes negativity of the ganglion with respect to the crushed postganglionic fibers. Traced from the original records projected by a photographic enlarger.

Fig. 2. Magnitude of the M2 spikes in response to a maximal test volley delivered at variable time intervals after a maximal conditioning volley, referred to the response to the test volley alone as a unit. Both volleys were obtained by stimulating the preganglionic nerve trunk through one pair of electrodes. Ordinates: spike magnitude. Abscissae: intervals between the two volleys in msec. Solid line, circles: before prostigmin. Broken line, dots: after prostigmin, 1.3 mgm. per kilogram.

is even more prominent if the time of appearance of the peak of the spikes, particularly that of M2, is measured; the increase may then be as great as 4 msec.

An increased delay is also present in the ganglionic records (fig. 1). A further difference is noticeable in that, although the magnitude of the spikes is not significantly changed, their duration is as a rule markedly increased (fig. 1). This apparent increase in duration may be explained

in part by the growth of the negative after-potential, as compared with the normal. The positive after-potential does not increase proportionately. Indeed, it is as a rule decreased, so that the responses show far more negativity of the ganglion with respect to the postganglionic fibers after than before the drugs. These effects are relatively long-lasting; they are still obvious up to two hours after injection of the higher doses mentioned.

II. *Responses to two shocks applied to the preganglionic nerve trunk.* The results of such an experiment on a normal ganglion have been discussed in detail elsewhere (Rosenblueth and Simeone, 1938). The changes elicited by eserine or prostigmin were consistent only when the test volley was applied at short intervals after the conditioning volley. These changes are typically illustrated in figure 2. The curve relating the responses to the test volley to the interval between the two volleys was invariably shifted to the right for the short intervals. For longer intervals the effects of the drugs were more irregular; in general, however, the curve obtained after the injections lay above the control curve—i.e., for a given relatively long interval the test response was usually greater after than before administration of eserine or prostigmin.

The responses to submaximal volleys are not uniform; hence considerable scattering appeared in the curves constructed from submaximal test volleys. For this reason conditioning and test maximal volleys were more frequently employed. The few experiments performed with submaximal test volleys agreed, however, in all respects with those in which the volleys were maximal.

III. *Responses to two shocks applied to the two branches of the annulus of Vieussens, respectively.* These experiments differ fundamentally from those reported in the previous section. There, the test volley traveled over preganglionic nerve fibers which had already responded to the conditioning volley. Here the two volleys traversed different preganglionic nerve fibers.

Barely liminal or submaximal stimuli (cf. Eccles, 1937) led in the present observations to responses which were so irregular as to render the results practically meaningless. For this reason we shall only report the interaction of maximal volleys at various time intervals.

Eccles' (1937) observation of marked variability for different cats in the distribution of preganglionic fibers in the two branches of the annulus was confirmed. Indeed, some animals had to be discarded because the M1 and M2 responses from one of the branches were quite small. The measurements were made mainly on the M2 spike, because the M2 preganglionic fibers were usually more uniformly distributed than the M1 fibers among the two branches.

In figure 3 (full line, circles) a typical curve is reproduced, showing

the variations in magnitude of the M2 spike to a maximal volley from one of the branches of the annulus, elicited at variable intervals after single stimulation of the other branch. The response to the test shock was measured after subtracting from the complex response to the two volleys the potential which the conditioning shock evokes when applied alone (cf. Eccles, 1935a). The validity of this method of measurement is discussed later (p. 716).

In 7 out of the 8 experiments performed (see table 1) an initial decrease of the response to the test volley was observed as the interval between the two volleys was increased (fig. 3). In the exceptional animal such an initial decrease did not occur (fig. 4).

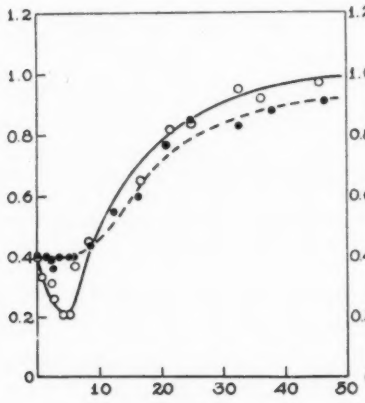


Fig. 3

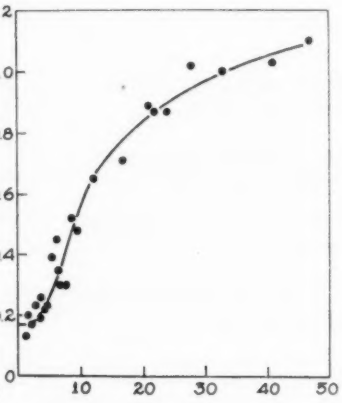


Fig. 4

Fig. 3. As in figure 2, except that the conditioning and test shocks were applied to one and the other branch of the annulus of Vieussens, respectively, and that eserine, 7.7 mgm. per kilogram, was used. Animal 1 in table 1.

Fig. 4. As in figure 3 before injecting any drug other than atropine. Animal 8 in table 1.

Injections of eserine or prostigmin led in the 7 typical cases to a reduction or a disappearance of the initial decrease of response with increasing intervals (fig. 3; table 1). In the atypical animal eserine had no influence on this early part of the curve.

The initial decrease is followed by an increase at longer intervals (figs. 3 and 4) so that the test responses attain or closely approach their normal magnitude at about 30 msec. Eserine or prostigmin invariably delayed this period of recovery (fig. 3; table 1).

The response to a test volley through a branch of the annulus is not only modified in magnitude but also in latency, when delivered at brief intervals after a conditioning volley from the other branch (Eccles, 1937).

The subtraction method employed for measuring the responses to the test volley was considered entirely inaccurate for determining the beginning of the spikes of the test response. The peak of the M2 spike, on the other hand, could be determined with an accuracy of about $\pm 1$ msec. and was found to vary consistently in all the experiments performed. Figure 5 illustrates a typical instance.

TABLE 1

| | | ANIMAL NUMBER | | | | | | | | AVERAGE |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | |
| | | Drug used | | | | | | | | |
| | | E 7.7 | E 7.5 | P 1.3 | P 0.9 | P 0.8 | E 6.1 | E 6.2 | P 0.7 | |
| Ratio of subtracted response to normal at interval 0 | B | 0.4 | 0.3 | 0.6 | 0.9 | 1.1 | 0.8 | 0.3 | 0.2 | 0.6 |
| | A | 0.4 | 0.3 | 0.6 | 1.0 | 1.0 | 0.8 | 0.4 | 0.1 | 0.6 |
| Ratio of minimal response to response at interval 0 | B | 0.6 | 0.6 | 0.6 | 0.7 | 0.8 | 0.7 | 0.0 | 1.0 | 0.6 |
| | A | 0.9 | 1.0 | 0.7 | 0.8 | 0.8 | 1.0 | 1.0 | 1.0 | 0.9 |
| Interval at which the minimal response occurred | B | 5.2 | 6.0 | 6.4 | 7.8 | 5.5 | 5.2 | 5.2 | | 6.0 |
| | A | 5.2 | 6.0 | 4.3 | 12.0 | 5.5 | 5.5 | 5.2 | | 6.1 |
| End of the rising phase succeeding the decline | B | 43 | 52 | 35 | 26 | 13 | 26 | | 26 | 32 |
| | A | 55 | 60 | 43 | 43 | 17 | 26 | | 30 | 39 |
| Maximal decrease of latency | B | 4.5 | | 4.3 | 1.3 | 3.1 | 1.7 | 3.4 | 5.3 | 3.4 |
| | A | 2.8 | | 5.1 | 1.6 | 3.4 | 2.6 | 1.7 | 4.9 | 3.1 |
| Interval at which latency was minimal | B | 5 | | 9 | 8 | 6 | 7 | 6 | 8 | 7 |
| | A | 6 | | 12 | 12 | 4 | 9 | 6 | 7 | 8 |

All the data refer to the M2 spikes of the test responses.

Abbreviations. E: eserine; P: prostigmin; the doses are expressed as milligrams per kilogram. B: before; A: after injection of the drug. The changes in magnitude of the test volley delivered alone (B and A) were never greater than 7 per cent; they averaged 0. The intervals are expressed in msec. The latencies refer to the peak of M2; they are expressed in msec.

The changes in latency with variable volley intervals were usually less prominent after eserine or prostigmin than before administration of the drugs. Table 1 summarizes this and other features of the experiments reported in this section.

IV. *Responses to repetitive stimulation.* The changes in spike magnitude, latency and pattern which take place upon repetitive stimulation of normal ganglia at different frequencies are described elsewhere (Rosenblueth and Simeone, 1938). Eserine or prostigmin modify strikingly the responses to repetitive stimulation.

The effects of the drugs upon the changes in magnitude of the spikes may be summarized as follows. With sufficiently high frequencies, de-

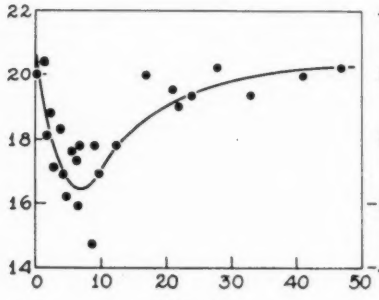


Fig. 5

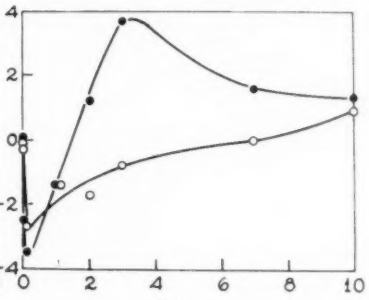


Fig. 6

Fig. 5. Ordinates: latency in msec. of the peak of M2 for the test responses, the magnitude of which is plotted in figure 4. Abscissae: intervals between the two volleys in msec.

Fig. 6. Changes in the latency of the spikes during repetitive stimulation at the rate of 30 per sec. after prostigmin, 0.3 mgm. per kilogram. Circles: M1. Dots: M2. Ordinates: changes in latency in msec. Abscissae: time in sec. after beginning of stimulation.

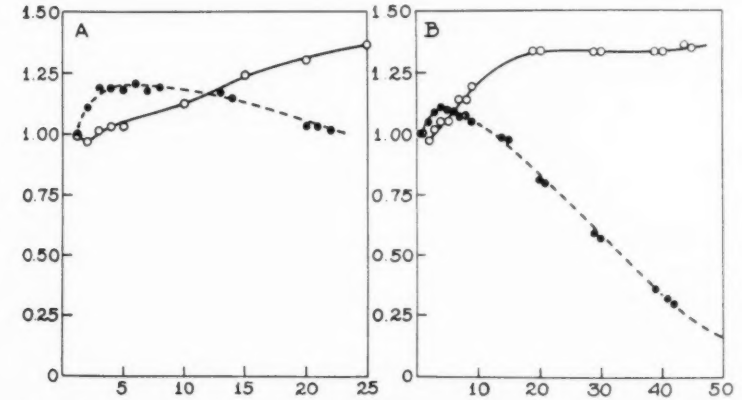


Fig. 7. Changes in the magnitude of M2 during maximal tetanic stimulation. Ordinates: magnitude of the spikes referred to the response to the first shock as a unit. Abscissae: number of shocks in the repetitive series. Solid lines (circles): before prostigmin. Broken lines (dots): after prostigmin, 1 mgm. per kilogram. *A*, frequency 10 per sec. *B*, frequency 19 per sec.

pending upon the doses injected, the responses decrease after a few shocks have been delivered; the decrease is first more rapid, then proceeds at a slower rate (fig. 7). Within limits, the greater the dose of the drugs the

slower the frequency at which the decrease of the spikes will be observed. For a given dose, the greater the frequency of stimulation the earlier and the more marked the decline of the spikes (fig. 7).

With moderate doses and with high frequencies of stimulation (30 to 90 per sec.) the initial decline of the spikes just described is succeeded, if the stimulus be prolonged, by a subsequent increase, and finally, later, by an ultimate decrease. Figure 8 illustrates a typical instance.

These effects of the drugs are transitory. With small doses (e.g., up to 2 mgm. per kgm., eserine, or 0.3 mgm. per kgm., prostigmin) they disappear within about 30 minutes after the injections. Further doses will again elicit the phenomenon (cf. Cannon and Rosenblueth, 1937). Larger doses have more enduring effects.

Both the early decrease and the late increase in latency of the spikes which occur in normal ganglia during repetitive stimulation become more prominent after injections of eserine or prostigmin. Figure 6 illus-

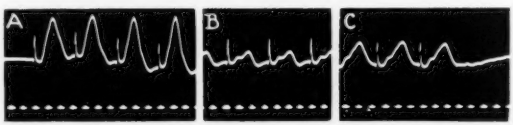


Fig. 8. Decline, followed by increase of the responses to repetitive stimulation. Frequency: 30 per sec. Ten minutes after prostigmin (0.3 mgm. per kgm.). The sharp excursions are the stimulus artifacts. The spikes M1 and M2 are clearly recognizable, especially in *B* and *C*. Upward excursions denote negativity of the caudad grounded electrode on the ganglion. Time: 10 msec. *A*.—Beginning of stimulation. *B*.—Four seconds later. *C*.—Twelve seconds later, end of stimulation.

trates an instance in which the latencies of M1 and M2 were decreased as much as 2.5 and 3.5 msec., respectively, early during repetitive stimulation at the rate of 30 per sec.

Repetitive discharges from some of the ganglion cells in response to the single nerve volleys during tetanic stimulation were probably present in some of the records obtained after injection of the drugs. The difficulties inherent in the interpretation of such records have been previously discussed (Rosenblueth and Simeone, 1938). Clear instances were found of after-discharge from the ganglion at the end of repetitive stimulation (cf. Eccles, 1936). Such after-discharge lasted on one occasion for 100 msec.

Discussion. If acetylcholine had only a stimulating action on ganglion cells and if eserine or prostigmin had no other effect on the ganglion than the protection of acetylcholine, then all the observations made thus far on the effects of these drugs on the transmission of the preganglionic nerve impulses would be crucial tests for the theory of chemical mediation of these impulses by acetylcholine. Acetylcholine, however, acts not only as a stimulant for the ganglionic neurones, but in higher concentra-

tions paralyzes these neurones both to further doses of acetylcholine and to preganglionic nerve impulses (Feldberg and Vartiainen, 1934). Eserine and prostigmin, on the other hand, may have a depressant effect on the ganglion (Feldberg and Vartiainen, 1934; Cannon and Rosenblueth, 1937; see below p. 717). The view that eserine should in any experiment lead to increased ganglionic responses (Eccles, 1935b, 1937) is, therefore, untenable. Indeed, the complicating factors are sufficient to render any negative experiments quite indiscriminative.

The striking influence of the drugs on the responses of the ganglion to repetitive stimulation (figs. 7 and 8; section IV) extend to the M2 group the observations previously made (Cannon and Rosenblueth, 1937) on the M1 elements which supply the nictitating membrane. The explanation suggested by Cannon and Rosenblueth still appears adequate: upon repetitive stimulation after eserine or prostigmin, acetylcholine accumulates at the ganglion until it reaches paralytic concentrations, hence the initial decline of the spikes; relative exhaustion of the output of acetylcholine after prolonged stimulation decreases the concentration back into the effective range, hence the delayed increase of the spikes.

The marked shortening of the synaptic delay which appears early during repetitive stimulation (fig. 6) is readily explained on the basis of the above suggestions. The quanta of acetylcholine liberated by the successive shocks are added to the remainder of the protected acetylcholine from the preceding volleys. Threshold concentrations will therefore be attained more rapidly than normally. The greatly increased delay after prolonged stimulation may be explained, as for the normal ganglion, by a decreased rate of liberation of acetylcholine and by changes in the threshold of the cells (see Rosenblueth and Simeone, 1938).

The slight increase of latency in the responses to single shocks (fig. 1) suggests that large doses of the drugs may raise the threshold of the cells to acetylcholine. No direct data are available to confirm or invalidate this suggestion. The increase of the negative after-potential (fig. 1, section I) may denote changes in the rate of recovery of the cells, since, as Gasser (1937) has suggested, the after-potentials are probably correlated with the recovery process.

The experiments concerning the influence of a conditioning volley on the response to a test volley applied at variable time intervals (sections II and III) require certain introductory considerations for their interpretation.

The method of subtraction used for measuring, in the complex records from the two volleys, the spike resulting from the test volley at short intervals does not yield the accurate information which Eccles (1935a, b and c; 1937) has assumed. Since the nerve elements discharge in parallel, the standard explanation for the growth of the potentials recorded

when the number of active elements increases is the progressive removal of shunting inactive fibers. In such a system, whether the nerve elements be considered analogous to batteries or to condensers, the responses are not lineally but less than lineally proportional to the number of fibers activated—i.e., activation of twice a given number of fibers yields less than twice the potential which the given number develops. Although no experiments were made on the ganglion or postganglionic fibers to test directly this deviation from lineal proportionality, some observations made on the peroneal nerve and on the preganglionic fibers showed that this deviation may be considerable. Stimulating electrodes were placed on the two branches of the annulus and recording electrodes on the preganglionic trunk. The responses to maximal stimulation of each of the branches, and of the two simultaneously, were recorded. The spike potential from either of the branches, measured by the subtraction method, was then found to be smaller by as much as 25 per cent than that recorded when the branch was stimulated alone. The results from two branches of the peroneal nerve were similar (30 per cent reduction in one case).

This source of error is important for interpreting some of the experiments on the ganglion. Eccles (1935a) made observations similar to those described in section III. The M2 response to one of the branches of the annulus, measured by the subtraction method, was found very small when the two branches were activated simultaneously. Eccles concluded that this small spike denoted great occlusion and hence that there was considerable overlap in the liminal fields of the two branches. It may be mentioned parenthetically that the present results (table 1) agree only exceptionally with those of Eccles. But the previous discussion shows that even when the responses are small in these conditions, a significant part of this apparent occlusion is illusory, due to the method employed for measuring the response.

The differences in the results obtained before and after injections of drugs, however, are independent of this source of error, since the method of measurement was the same in the two conditions and since the time course of the responses to the conditioning volleys was only slightly affected by the drugs.

In the experiments on two maximal shocks delivered with variable time intervals to the preganglionic trunk (fig. 2), the second shock fails to elicit any response if applied less than 3 to 4 msec. after the conditioning volley. This inability to excite is due first to the absolutely refractory period, then to the early part of the relatively refractory period of the preganglionic fibers and the ganglion cells. The responses later increase gradually to approach or reach their normal size usually when the test volley is applied from 25 to 40 msec. after the first. These increasing responses furnish probably a measure of the relatively refractory period

of the different cells in the M2 group (see p. 718). After having reached full value at those intervals the responses may continue growing or may show a late decline at longer intervals. This later part of the curves has been discussed elsewhere (Rosenblueth and Simeone, 1938).

The early depression due to refractoriness, absolute and relative, is augmented and prolonged after eserine or prostigmin (fig. 2). It may be concluded that the drugs slow the recovery process, a direct effect which is probably unrelated to the protection of acetylcholine. Apart from revealing a depressant action of the drugs on the ganglion, the experiments of section II do not yield any unequivocal information with respect to the processes occurring at the ganglion. When submaximal test shocks are used and an increase of the responses is observed at certain intervals, the possibility of "facilitation" is suggested (Eccles, 1935b). Since, however, maximal test volleys elicit similarly increased responses (fig. 2), it can be inferred that this increase may at least partly be due not to synaptic processes, but to changes in the postganglionic elements leading to greater spike potentials (cf. Rosenblueth and Simeone, 1938). The usual increasing action of the drugs on the responses falling in this region of the curves (p. 710) would then correspond again to a direct effect on the postganglionic elements, not to protection of acetylcholine.

For the interpretation of the experiments in which the two branches of the annulus are stimulated (figs. 3 and 4; table 1) further introductory considerations are necessary. In the ganglion the fields of distribution of the fibers of each of the branches may overlap in certain regions and be independent in others. The regions where there is an overlap may be further subdivided as follows: (*a*) cells to which both branches deliver liminal impulses—i.e., cells which respond to either of the branches; (*b*) cells to which one branch is liminal, the other subliminal; (*c*) cells to which both branches deliver only subliminal impulses—i.e., which respond only when impulses from the two branches reach them within adequate time intervals. If the two branches are stimulated simultaneously area *a* will lead to occlusion of the responses, area *c* will lead to facilitation, and the two opposite influences will coexist. Hence, even if accurate methods for measuring the responses were employed, an increase of the response to the test volley would not necessarily exclude occlusion, but might denote a preponderance of facilitation over occlusion; and conversely, a decrease would denote a preponderance of occlusion.

On this basis the results before injection of the drugs in the first 7 experiments in table 1 (cf. fig. 3) may be explained as follows. The responses to the test volleys were sometimes smaller, sometimes larger, when delivered simultaneously with the conditioning volley than when delivered alone—i.e., occlusion or facilitation preponderated in different experiments. Indeed, since the values for the subtracted responses upon simultaneous

stimulation are lower than they would have been with a more accurate method of estimation (p. 716), table 1 gives an undue emphasis to occlusion and underestimates facilitation.

As the interval between the conditioning and the test volleys is lengthened the test response decreases, to be minimal at about 6 msec. This decrease should denote the decay of the process responsible for facilitation of subliminal impulses at simultaneity. Eccles (1937) speaks of this process as the "detonator" response of the cells. For present purposes the process will be designated as the decay of the *mediator* of the nerve impulses in the areas where facilitation occurs.

At longer intervals the responses suddenly begin to increase, first more rapidly, then more slowly. This increase need not coincide with the end of decay of the mediator, since at those intervals there should be a progressive recovery from refractoriness of the cells in the occlusion area. This interpretation leads to values of about 6 msec. for the absolutely refractory period of the M2 group (cf. Eccles, 1935a) and of about 30 msec. for the relatively refractory period. These values are in agreement with those deduced from the experiments of section II (p. 716).

In all 8 experiments, the test responses increased at intervals of from 6 to 30 msec. It follows then that in all cases there was a significant degree of occlusion. In 7 animals an early decline appeared and in 1 (fig. 4) it was absent. It follows that in the exceptional animal there was little if any area where facilitation was necessary for the cells to discharge (area *c*).

After eserine or prostigmin the early decline of the test responses was considerably reduced or disappeared altogether (fig. 3; table 1). If the previous suggestion is accepted, that this decline is a measure of the rate of decay of the mediator of the nerve impulses, the conclusion follows that the drugs slow this rate—precisely the conclusion that could be predicted if acetylcholine is the mediator in question.

At longer intervals the responses are smaller after than before injection of the drugs. Since this segment of the curves measures the recovery from refractoriness of the cells in the occlusion area the inference follows that the drugs slow the recovery of the cells (cf. p. 717).

The changes of latency in the annulus experiments (fig. 5) are of interest. The decrease at short intervals may be explained as follows. Obviously the only cells in which such a shortening can take place are those in the areas where the two volleys overlap, for the conditioning volley could have no influence on the cells supplied exclusively by the test volley. Obviously also the decline of latency at intervals shorter than the absolutely refractory period of the cells excludes the area *a* of occlusion (p. 717) and the area *b*1, where the conditioning volley is liminal and the test volley subliminal. The shortening then should be due to an early

discharge of cells in area *c* (both volleys subliminal) and area *b*2 (conditioning volley subliminal, test volley liminal). Indeed, experiment 8 (fig. 4) proves that area *b*2 exists, for in that case there was probably no area *c* (p. 718), yet a typical shortening of latency was observed (table 1).

The decreased latency may be partly explained by assuming that the mediator to the test volley reaches threshold sooner than normally, because it adds to the mediator already liberated by the subliminal conditioning volley. According to this explanation eserine or prostigmin should prolong the period of decreased latency. In the present observations they failed to do so. Not much emphasis should be laid on this failure, because (the measurements not being very accurate) slight changes would fall within the limits of experimental error, and because the measurements were made not on the shortest latencies of the spike, but on the times for attainment of a peak—i.e., a rough indication of the average latencies. That the drugs may affect significantly synaptic delay is shown by the marked shortening of latency early during repetitive stimulation (fig. 6).

The previous explanation of the shortened delays in the annulus experiments differs from that suggested by Eccles (1937) in only one respect. Instead of assuming a summation of "detonator" responses we assume accumulation of acetylcholine, the chemical mediator. Eccles suggests that the "detonator" response begins only after a measurable delay (2 msec. for the M1 elements). This suggestion rests on the fact that the synaptic delay in his two-shock experiments could never be reduced to less than that interval. Since, however, upon repetitive stimulation after prostigmin the synaptic delay may be reduced to a fraction of a millisecond (cf. fig. 6), it seems more reasonable to assume that the chemical mediator begins to be liberated almost instantaneously at the arrival of the preganglionic nerve impulses.

The failure of the electrical theory to account adequately for transmission at the ganglion has been repeatedly pointed out (cf. Cannon and Rosenblueth, 1937; Rosenblueth and Simeone, 1938). Eccles (1936, 1937), who defends electrical transmission, has been compelled, in order to account for the data, to introduce a hypothetical "detonator" response between the arrival of the nerve impulse and the discharge of the cells. This "detonator" response he homologizes with the local excitatory state of nerve (1937); but there is no experimental basis for this homology, since the "detonator" is exclusively defined by the discharge of the cells (1937, p. 11). From the present experiments (fig. 3) it would follow that the "detonator" response would have to be prolonged by eserine or prostigmin; there is no evidence that the local excitatory state is similarly influenced. The chemical theory, on the other hand, accounts readily for the phenomena through use of only experimentally established premises (p. 718). The failure of the drugs to affect significantly the changes of

latency of the peak of the test spikes in the annulus experiments (fig. 5), while increasing clearly the magnitude of test responses at short intervals (fig. 3), would meet with identical difficulties of explanation whether a "detonator" response or a chemical mediator be assumed as intermediary between the preganglionic nerve impulses and the discharge of the cells.

Eccles (1935b, 1937) made three series of experiments to test the acetylcholine theory of transmission by means of eserine: (*a*) two shocks (the second usually submaximal) to the preganglionic trunk; (*b*) two submaximal volleys through the branches of the annulus; (*c*) two maximal volleys on the preganglionic and postganglionic fibers, respectively, the nictitating membrane being the indicator. He obtained negative results with all three tests. In the present observations the experiments mentioned under *a* and *b* above were repeated. Maximal volleys were used, because submaximal stimuli led to inconsistent responses (sections II and III). The experiments of section II (see *a* above) do not appear especially suited to test discriminatively the acetylcholine hypothesis (p. 717). The experiments of section III (see *b* above) yield unequivocal supporting evidence of chemical mediation (p. 718). As mentioned in the introduction, Eccles' (1937) negative results were probably due to insufficient doses of eserine (0.1 to 1 as opposed to 4 to 8 mgm. per kgm. in the present observations). We attempted to repeat Eccles' (1937) experiments listed as *c* above in three animals, but abandoned the procedure for the following reasons. Large doses of eserine or prostigmin require injections of atropine to eliminate seriously inconvenient manifestations of parasympathetic hyperactivity. After injections of atropine the responses of the nictitating membrane to single nerve volleys usually become too small for accurate measurement. Besides this technical difficulty it soon became evident that maximal stimulation of the intact postganglionic fibers would probably activate simultaneously at least some of the preganglionic fibers at the ganglion by spread of the very strong shocks necessary. This probable spread of the conditioning stimulus to the fibers activated by the test volley would introduce uncontrolled complexities into the experiment, so that neither positive nor negative results could be satisfactorily analyzed.

## SUMMARY

The influence of eserine or prostigmin on the superior cervical ganglion was studied in cats as follows: responses to single preganglionic volleys (section I, fig. 1); responses to two shocks applied to the preganglionic nerve trunk (section II, fig. 2); responses to two shocks applied to one and the other of the two branches of the annulus of Vieussens, respectively (section III, table 1, figs. 3, 4 and 5); responses to repetitive stimulation (section IV, figs. 6, 7 and 8).

The results are discussed with reference to their bearing on the theory of chemical mediation of the preganglionic nerve impulses by acetylcholine. The drugs slow the rate of decline of the mediator of the nerve impulses (p. 718). This evidence supports the chemical theory. The drugs have also a depressant action on the ganglion cells (p. 717). The negative results reported by Eccles (1935b, 1937) are discussed on the basis of the present observations and inferences (p. 720).

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# THE RELATION OF FOOD INTAKE TO THE DEVELOPMENT OF PARATHYROID TETANY IN THE RAT

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Received for publication January 28, 1938

Numerous observations have indicated that the rat does not develop tetany following the removal of the parathyroid glands with the regularity of some other species, especially the dog. Some rats will experience violent tetany within a day or two following parathyroidectomy, while others under apparently identical conditions will show no signs of tetany. The reasons for these divergent responses have never been entirely explained. Shelling (1) believes that the primary factor in determining the reaction of the rat to a loss of parathyroid function is the level and ratio of calcium and phosphorus in the diet. He found that a high calcium to phosphorus ratio will prevent the onset of tetany whereas a low ratio will cause tetany to become manifest. Hoskins (2), however, has been unable to confirm entirely these observations. Shelling has also emphasized the frequency with which tetany is accompanied by a marked decrease in food consumption. He is of the opinion that the apparent loss of appetite is the result of the attacks of tetany. However, as we have previously pointed out (3) the decreased food intake, in some cases at least, may be a contributing cause and not the result of tetany. When little or no food is being consumed there is obviously a deficiency of calcium, and in addition there is a tendency to a rise in inorganic phosphorus within the body due to the oxidation of phosphorus-containing organic compounds. Both of these conditions tend to increase the severity of tetany. In her report Hoskins made no mention of food consumption nor of the level of inorganic phosphorus of the serum. She did find that rats which were sickly or underweight were more likely to develop tetany than healthier animals, and that most deaths which were caused by the loss of the parathyroids occurred within 48 hours after the removal of the glands. It is possible that the rats dying within this period ate little or no food subsequent to the removal of the parathyroids. It is also a common observation that the highest incidence of tetany is within this same period of time. Thus it appears that on diets with a fairly high calcium to phosphorus ratio sturdy animals which can undergo the surgical operation with little immediate

after-effect soon regain their appetites and can ward off the onset of tetany. In contrast to this, individuals which are sickly or underweight recover more slowly from the shock of the operation with a consequent prolonged anorexia which in turn exaggerates the conditions tending to produce tetany. In the following experiments the relation of food-intake to the development of tetany has been studied more in detail.

EXPERIMENTAL. In the first series of experiments both adult and young rats were used. The parathyroid glands were removed under ether anesthesia. Only those animals (total of 61) from which both the glands were definitely identified after removal as previously described (4) were taken for study. Following the ablation of the glands the animals were all given the dry portion of the Steenbock (5) stock diet containing an additional 1.0 per cent $CaCO_3$. This diet is fairly high in calcium, contains a moderate amount of phosphorus and has a calcium to phosphorus ratio of approximately 1.7. Daily food consumption records were kept. Several of the young animals had been raised in connection with studies on reproduction. They were somewhat underweight and not very sturdy. These animals were used for the purpose of comparing the present results with earlier experiments in which it was shown that young rats from our regular stock could withstand the removal of the parathyroids with practically no incidences of tetany or death providing the diet used contained liberal amounts of calcium. Seven of these rats died within 24 hours after loss of parathyroid function. None of them had consumed any food during this period, consequently the diet offered could have no bearing on the outcome. Most of the remaining animals ate the food well and showed no signs of tetany as long as they were continued on this diet. However, the food intake in a few cases, especially with some of the adults, progressively decreased until little or no food was being consumed. Just half of the animals with a decreased food-consumption showed tetany of varying severity when the amount of food being eaten was reduced to approximately 3 grams or less per day, and two of them died. Food consumption of the others increased about the 10th day and rapidly returned to normal with a complete disappearance of the tetany symptoms.

After 2 to 3 weeks on the high-calcium diet the 52 surviving animals were divided into 3 groups (Groups I, II, III—table 1). Group I was continued on the same diet, group II was deprived of any food and Group III was given pure sucrose. The latter group was included with the hopes that it might be possible to throw some light on the part phosphorus plays in determining the level of serum calcium and the onset of tetany. If the rise in the inorganic phosphorus of the serum is due to excessive oxidation of phosphorus-containing compounds within the body then it should be possible to prevent, at least partially, this oxidation by simply supplying a source of energy.

All three groups of animals were killed by bleeding 24 hours after the changes in the diets were made. The sera were analyzed for calcium by the method of Clark and Collip (6) and inorganic phosphorus was determined on the calcium-free filtrates by the method of Gunther and Greenberg (7). When sufficient serum was available a check phosphate determination was done directly on the serum by the method of Fiske and Subbarow (8). In each case, however, the value given is that obtained on the filtrate.

The results are summarized in table 1, section A. In a few instances not enough serum was obtained from each animal for individual analyses. In these cases the sera from 2 or more animals were united. In table 1 are

TABLE 1

*Influence of diet and food intake on tetany and composition of serum of parathyroidectomized rats*

| GROUP NUMBER | EXPERIMENTAL DIET | NUMBER OF ANIMALS | NUMBER SHOWING TETANY | NUMBER OF ANALYSES | SERUM | |
|---|---|---|---|---|---|---|
| | | | | | Ca | P |
| A. Animals fed high-calcium diet for period following removal of glands | | | | | | |
| | | | | | *mgm. per 100 cc.* | *mgm. per 100 cc.* |
| I | High-calcium (control) | 16 | 0 | 13 | 9.4 | 7.4 |
| II | No food | 18 | 13 | 14 | 6.3 | 13.2 |
| III | Sucrose | 18 | 4 | 16 | 7.8 | 10.8 |
| B. Animals fed experimental diet immediately following removal of glands | | | | | | |
| IV | High-calcium (control) | 9 | 1 | 8 | 10.0 | 6.3 |
| V | No food | 7 | 5 | 7 | 6.9 | 9.2 |
| VI | Sucrose | 4 | 0 | 4 | 7.9 | 7.7 |
| VII | Low Ca-low P* | 6 | 1 | 6 | 9.1 | 5.8 |

* Dextrin, 81 per cent; alcohol extracted-acid washed fibrin, 18 per cent; NaCl, 1 per cent.

given the number of animals comprising each group and also the number of analyses performed. The results given for calcium and phosphorus are the averages of all determinations of each group.

When the results obtained from group I are compared with those from Group II it can be seen readily what a pronounced effect the deprivation of food has on the blood composition and development of tetany in parathyroidectomized rats. Of the 16 animals which were continued on a high calcium diet none showed tetany during the 24-hour experimental period, whereas of the 18 which were deprived of food 13 were observed in tetany within this time.

The composition of the serum of the other 5 of this group was not far

from that characteristic of animals in tetany. In each case the calcium was below 8 mgm. and the phosphorus above 10 mgm. per cent. These animals probably would have given some indication of tetany had they been continued on the fasting regimen for a longer time. The proportion developing tetany following the sudden withdrawal of food was considerably greater than that which showed tetany during the previous period of decreased food consumption. In respect to the animals receiving sucrose it appears that the simple supplying of energy afforded a certain degree of protection when compared to the animals receiving no food as indicated both by the incidence of tetany and the levels of calcium and phosphorus in the serum. The protection was far from being as complete as it was on the high calcium diet given to the control animals. This result is somewhat in disagreement with previous work by the author (3) in which it was shown that parathyroid tetany in the rat could be prevented by making the phosphorus of the food unavailable even though there was no calcium in the diet. In the present case, however, there was no protein in the ration which would probably lead to an abnormal destruction of body tissue, with the consequent rise in serum phosphorus, even in the presence of an ample supply of energy. Furthermore, some of the animals did not eat very much of the sucrose which would result in partial starvation.

In a second series of experiments, in which 35 young and adult rats were used, the animals were placed on the experimental diets immediately after the parathyroids were removed and blood samples were taken, as above, 24 hours later. The diets used were the same and in addition 6 of the adult animals were given a diet somewhat more complete and more palatable than simple sucrose but containing only traces of calcium and phosphorus. This diet was composed of dextrin 81 parts, sodium chloride 1 part and fibrin 18 parts. The fibrin was made practically free of calcium and phosphorus by extracting with alcohol and washing with dilute acid.

The results obtained from this series of experiments, Groups IV, V, VI, and VII (table 1, section B), are very similar to those recorded above. Of 11 young animals subjected to parathyroidectomies 3 were given no food, 2 were given the high calcium diet and the remaining 6 received sucrose. Twenty-four hours later all but the 2 receiving the high calcium diet were dead and one of the latter was in tetany. This animal had consumed but 1 gram of food during the 24 hours and 3 grams were the maximum consumed by any of those given sucrose. No blood was obtained from the animals which died and consequently they are not included in the averages given in table 1.

None of the adult animals which received the high calcium diet was observed in tetany and the calcium and phosphorus of the sera were only slightly altered. Five of the 7 older animals which were deprived of food

were in tetany the following morning. The highest calcium level in this group was 8.1 mgm. per 100 cc. of serum which was given by one of the animals not seen in tetany. The serum phosphorus in this case was the lowest of any of this group, it being 6.1 mgm. per cent. Only 4 adult rats of this series were given sucrose. None of them was observed in tetany but the serum calcium of each was considerably below normal and the phosphorus was somewhat above.

One of the animals fed the fibrin-containing diet was observed in tetany the following morning. However, this rat consumed none of its diet subsequent to the removal of the glands. The serum calcium was the lowest and phosphorus the highest of any of this group. None of the other animals receiving this diet showed any tetany, and the calcium and phosphorus of the sera were within normal limits.

This same experiment was repeated later with 6 other adult rats (results not included in the table) which, like some of the younger animals previously mentioned had been used for other purposes and were not in the best condition. Of these 6 animals 4 had a low serum calcium and high serum phosphorus 24 hours after the removal of the parathyroids. None of these four animals (2 of which showed tetany) consumed any food following the removal of the parathyroids. Each of the other two animals ate 6 grams of food during the 24-hour experimental period. They both appeared normal and the concentrations of serum calcium and phosphorus were within normal limits. It appears that the diet containing protein although very low in calcium and phosphorus afforded better protection against tetany than did a simple source of energy such as sucrose. However, sucrose alone afforded more protection than complete deprivation of food.

It has been frequently observed that tetany is less likely to appear if the calcium of the serum falls slowly than if it falls rapidly, although the level reached may be lower in the former case than in the latter. In view of the results obtained when the animals were suddenly deprived of food it was thought that probably different results might be obtained if the food given were reduced gradually. With this in mind the parathyroids were removed from 12 adult female rats and the stock diet with the added calcium previously used was fed. Six of these animals showed the gradual decrease in food intake described above. Instead of continuing them on this diet until the consumption of food increased they were killed by bleeding when the intake of food was 2 gms. or less per day. Although the serum calcium levels were between 5.2 and 7.7 mgm. and the phosphorus between 9.7 and 13.0 mgm. per 100 cc. of serum only one of these 6 rats showed any tetany and that was only during the taking of the blood. The other 6 of this group of 12 rats continued to eat and remained free from tetany. Starting on the 10th day following the removal of the parathy-

roids the amount of food allowed each individual was decreased by one gram per day until no food was being given. After 24 hours with no food-allowance the animals were bled. Each one of these 6 animals showed tetany at some time or other before being sacrificed. Usually tetany first appeared when 3 or 4 grams of food per day were being given. In some cases it was observed periodically until the animal was killed. At no time was there any apparent decrease in appetite. Even during an attack of tetany the animals were active and seemingly in search of food, and as soon as any was given the animals would go to the feeding-cup and eat the food allowed. The sera of these 6 animals all had the low calcium and high phosphorus characteristic of tetany. Thus it is doubtful if anorexia is necessarily a result of tetany.

Greenwald (9) and Hoskins (2) have expressed the belief that following parathyroid removal some adjustment takes place which tends to prevent tetany. They believe that if the calcium falls slowly and the onset of tetany is delayed, this compensatory mechanism has time to come into play, whereas if the calcium falls rapidly the adjustment cannot take place and tetany results. It is apparent from the data presented above that if there is such a mechanism the simple removal of the parathyroids does not bring it into action. If rats from which the glands have been removed are protected against tetany for several weeks by feeding a diet with a fairly high calcium to phosphorus ratio they will nevertheless develop tetany within 24 hours if deprived of food. Furthermore, if the conditions tending to produce tetany are brought on slowly by gradually reducing the food allowance tetany is still not prevented. If there is a special mechanism for the prevention of tetany in the absence of the parathyroid glands, it should, in either of the above cases, have had time to exert its influence.

## SUMMARY AND CONCLUSIONS

The feeding of a stock diet with a calcium to phosphorus ratio of 1.7 to parathyroidectomized rats protected most of the animals against tetany. However, a few of the less sturdy ones died within 24 hours and the food consumption of some of the others gradually decreased and tetany resulted. After about 10 days food consumption returned to normal and tetany disappeared. If, after a period of 2 to 3 weeks on the high calcium diet, the animals were deprived of food most of them developed marked tetany within 24 hours. The feeding of sucrose as the sole dietary constituent afforded partial protection compared to fasting.

The majority of rats placed on a fasting diet immediately following the removal of the parathyroids developed tetany within 24 hours. The incidence of death was high especially among the younger animals. Sucrose gave a certain degree of protection but not as much as a more palatable diet containing protein but only traces of calcium and phosphorus. If

the conditions tending to produce tetany were brought on slowly by a gradual decrease in the food-allowance tetany was still not prevented.

The above data emphasize the importance of food intake as a factor in the development of parathyroid tetany in the rat. It also appears that the simple loss of parathyroid function does not, of itself, give rise to a compensatory mechanism which tends to prevent the development of tetany.

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# PANCREATECTOMY IN THE GOAT

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Received for publication January 29, 1938

The influence of species upon the metabolic changes which follow the removal of the pancreas was first studied by Minkowski (1). However, no reference to the study of pancreatic diabetes in the goat has been found although Lusk (2) studied phlorhizin diabetes in goats. Since the response to pancreatectomy in the pig (3) differed from that seen in the dog and cat, a few observations on caprine diabetes were undertaken. The results afford another instance of a "mild" form of diabetes following pancreatectomy.

METHODS. Young goats from 5 to 15 kgm. were used. Due to the large antero-posterior diameter of these animals even when very young, pancreatectomy presented certain surgical difficulties. The pancreas in the goat is closely related to the portal vein and lies upon the spine. It is compact and can be removed grossly. Some operations were done through a midline incision with removal of the organ in the usual manner. Except in the smallest animals, however, it was found easier to do a two stage operation through the costo-vertebral angles. The portal and duodenal portions could be quite readily removed by a first operation through the right flank. The splenic tip could later be removed through the left flank. Of 7 goats which survived operation, 4 had no demonstrable pancreas at autopsy. Nembutal anesthesia was used, the dosage being 40 mgm. per kgm. The animals were kept in metabolic cages and were studied as in the case of the pig (3). No insulin was given to these animals.

RESULTS. Table 1 summarizes the urinary excretion of glucose, nitrogen and acetone bodies during fasting, and the terminal serum chemistry of the 4 successfully operated animals. The low glucose and nitrogen excretion is comparable to the values found in the pig (3). Hutchinson and Morris (4) give figures for the nitrogen excretion of fasted goats, from which it has been calculated that the average fasting N is about 0.13 gram per kilo per day. Lusk (2) records almost identical figures. It is thus apparent, as in the case of the pig, that although the nitrogen excretion of the diabetic goat was very low it was more than doubled by pancreatec-

tomy. In contrast to the pig and to the carnivora, however, the excretion of acetone bodies was very low. Acetonuria was usually absent although small amounts appeared at times.

Survival was not studied in these animals. One (goat 4) died 44 days after pancreatectomy. The others were killed 9, 14 and 22 days after operation, while in apparently good health. The value for the liver fatty acids in goat 2, 9 days after pancreatectomy, was 0.42 per cent.

Weight loss occurred in all animals in spite of feeding, the loss being 5.6, 3, 2 and 3 kilograms in the order listed in table 1.

The animals were somewhat wild, and blood sugars were not routinely taken. Of 10 fasting blood sugars the range was 58 to 194 with an average of 124. The blood sugar of normal goats is 40 to 60. The word fasting is used with the knowledge that the goat's stomach does not empty. The animal with a blood sugar of 194 had no glycosuria for that day.

TABLE 1

*The fasting glucose, nitrogen and acetone body excretion and terminal serum chemistry of depancreatized goats*

| NUMBER | DAYS AFTER PANCREATECTOMY | URINE | | | TERMINAL SERUM CHEMISTRY | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Glucose | Nitrogen | Acetone bodies | Glucose | $CO_2$ | Urea N | Fatty acids | Total base | Chloride |
| | | *gram per kgm. per day* | *gram per kgm. per day* | *mgm. per kgm. per day* | *mgm. per 100 cc.* | *vol. per cent* | *mgm. per 100 cc.* | *m. eq. per l.* | *m. eq. per l.* | *m. eq. per l.* |
| 1 | 1-4 | 0.37 | 0.45 | 0 | | 50 | 63 | 0.08 | 143 | 104 |
| 2 | 1-3 | 0.11 | 0 21 | 4 | 165 | 47 | 33 | | 147 | 106 |
| 3 | 5 8 | 0 | 0.44 | 36 | 32 | 63 | 53 | 0.09 | | |
| 4 | 6-9 | 0 | 0.44 | 0 | | | | | | |
| Average.... | | 0.12 | 0.38 | 10 | | | | | | |

It appears that the normal goat has a blood sugar lower than the dog or man, but that its renal threshold may be fairly high. Of the blood chemistry studies (table 1), the values for $CO_2$ indicate the absence of marked acidosis, the urea nitrogen is increased as in other species, fatty acids are low (normal?) and chloride and total base are not depleted. The last finding may be attributed to the lack of glycosuria and diuresis.

Figure 1 illustrates the results of sugar tolerance tests. Curves A, B, C and D show the failure of glucose by stomach tube to influence the peripheral blood sugar. In one animal the abdomen was opened during the test and the duodenum and intestines aspirated. The contents were negative for sugar. At the end of the experiment the animal was sacrificed and the stomach washed out. No sugar could be found. The assumption is made that glucose is fermented or otherwise transformed in the herbivorous stomach and absorbed in some non-reducing form. The intra-

venous tolerance test E shows a more rapid tendency to return to the initial level than one would expect in a depancreatized animal. This animal (goat 4) only excreted 50 per cent of the injected glucose. Goat 3, weighing 4.6 kgm., was given 5 grams of glucose intravenously of which only 1 gram appeared in the urine during the subsequent 24 hours. Of the animals given glucose by stomach tube, three were sugar free in the following 24 hours, the fourth had some glucose in the urine which was attributed to gastric regurgitation in the cage shortly after the administration of the glucose.

Although the tests suggest that there was some utilization of glucose in the depancreatized goats, the nitrogen excretion was not diminished by glucose. It was not possible to observe any effect of food on the nitrogen excretion during the periods when the animals were fed.

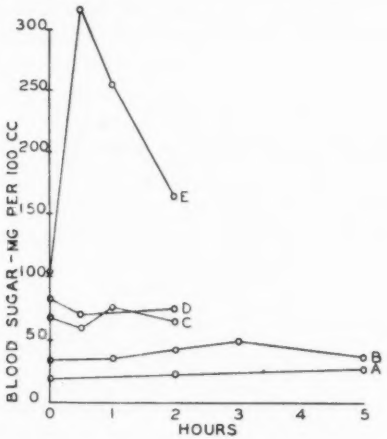


Fig. 1. Glucose tolerance curves of goats (under nembutal). *A* and *C* partially depancreatized; *B* normal; *D* goat 4 (depancreatized). These received 3 grams of glucose by stomach tube after the initial blood sugar. *E*, goat 4, given 1.77 grams per kgm. of glucose intravenously. See text for urine glucose during these tests.

Discussion. Following pancreatectomy, the goat has a mild type of diabetes as measured by the extremely low glycosuria and low, although increased, nitrogen excretion. In these respects it differs from the depancreatized cat or dog, and resembles the condition observed in the pig. Unlike the cat, dog and pig, however, the ketonuria is slight or absent. With the excretion of glucose, nitrogen and acetone bodies all diminished, the picture of pancreatic diabetes in the goat is extremely similar to that seen in the hypophysectomized-depancreatized dog or cat. A comparison has been made elsewhere of the influence of species and operative procedures upon pancreatic diabetes (3). A picture apparently similar to that of the goat has been reported in the depancreatized monkey (5).

Unlike any of the experimental animals previously cited, the depancreatized goat manifests a certain ability to utilize carbohydrate, as shown by the two animals in which glucose was given intravenously, and only 25 to 50 per cent excreted. The following explanations have been considered: 1. In spite of its compact pancreas, this species may have accessory pancreatic tissue which escaped careful search at autopsy. 2. The goat did not exhibit a rise in blood sugar following the introduction of glucose into the stomach and it is possible that carbohydrate derived from cellulose is utilized without going through the stage of glucose as in other animals. 3. The glucose injected intravenously and not excreted by the depancreatized goat only amounted to 1 gram per kilo per day in these two tests. This occurred in addition to the utilization of the carbohydrate derived from protein. The calculation of endogenous carbohydrate formation from urine nitrogen has not been attempted since it would require an arbitrary choice between the actual and the classical D:N ratios.

TABLE 2

*Urine glucose, nitrogen and D:N ratios in pancreatic and phlorhizin diabetes*

| NUMBER AND SPECIES | AVERAGE VALUES | | D:N | PROCEDURE |
|---|---|---|---|---|
| | Glucose | Nitrogen | | |
| | *gram per kgm. per day* | *gram per kgm. per day* | | |
| 5 pigs | 0.2 | 0.5 | 0.4 | Depancreatized (3) |
| 1 pig | 1.1 | 0.5 | 2.2 | Phlorhizinized (9) |
| 4 goats | 0.1 | 0.4 | 0.3 | Depancreatized |
| 2 goats | 0.8 | 0.3 | 2.7 | Phlorhizinized (2) |

With regard to the completeness of the pancreatectomy, the findings in phlorhizin diabetes in this species may be cited (2). Lusk (6) regarded phlorhizin diabetes as a means of confirming the completeness of Minkowski's pancreatectomies because the D:N ratio in the two types of diabetes were fairly similar in the case of the dog. Lusk (6) has compared pancreatectomy and phlorhizin diabetes in the dog in detail.

In table 2 the comparison is made between these two types of experimental diabetes in the goat. The pig is included because it illustrates the same type of response as the goat. In these two species the findings in pancreatic and phlorhizin diabetes do not run parallel as they do in the dog or cat. The glucose excretion and the D:N ratios differ considerably, both being lower in pancreatic diabetes. It is therefore noteworthy that the nitrogen excretion is increased identically in both types of diabetes. One may suppose that pancreatectomy in the goat has

been complete, since it produces the same nitrogen excretion as phlorhizin. The difference in glucose excretion may then be attributed to one or more of three mechanisms: 1. Different proportions of carbohydrate may be derived from protein under the two experimental conditions. Lusk (6) suggested this to explain slight differences between phlorhizin and pancreatectomy in the dog. If this be the explanation, the difference in the amount of carbohydrate derived from protein under the two experimental conditions is tenfold in the pig and goat. 2. Glucose may be converted to fat in pancreatic diabetes, but not in phlorhizin diabetes. The evidence is inadequate as to this. 3. If the tissues utilize traces of glucose in the absence of insulin *a*, the percentile utilization will appear larger where the supply (indicated by urine N) is small. The nitrogen output of the pig and goat, in spite of its increase during diabetes, is considerably lower than that of the diabetic dog. *b*. The utilization of glucose is favored by a high blood sugar level (7), even in the absence of insulin (8). Elevation of the blood sugar occurs in pancreatic diabetes and not in phlorhizin diabetes, and this combination of a high blood sugar and a low rate of gluconeogenesis appears to be the most likely reason for the difference between these two types of diabetes.

The possibility that an altered degree or type of pituitary or other endocrine function is present may be considered as a cause of the low nitrogen excretion, but has not been studied by pituitary extract injections.

## SUMMARY

Four young goats have been depancreatized. The fasting nitrogen excretion was low; the glucose and acetone body excretion was slight or absent.

The results have been compared with previous studies in other species and with phlorhizin diabetes in the pig and goat.

Although the nitrogen excretion of the goat is identical in phlorhizin and pancreatic diabetes, the glycosuria of the two experimental conditions differs widely. The increase in blood sugar after pancreatectomy combined with the low rate of gluconeogenesis is regarded as the most probable explanation of this situation.

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# NUTRITIONAL REQUIREMENTS FOR NORMAL GROWTH AND REPRODUCTION IN RATS STUDIED BY THE SELF-SELECTION METHOD

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Received for publication February 3, 1938

The ability of animals and humans to make dietary selections which are conducive to normal growth and reproduction has not received much attention in modern nutrition studies. The survival of animals and humans in the wild state in which the diet had to be selected from a great variety of beneficial, useless, and even harmful substances is proof of this ability. It would be of interest to know the ingredients of this self-selected diet; the percentage of fat, carbohydrate, and protein, and the mineral and vitamin content. This knowledge would probably throw an entirely new light on the nutritional requirements for growth, health, and reproduction.

In the wild state, quantitative studies of the food intake of animals and humans would be impractical. It is necessary, therefore, to try to reproduce the essential features of field conditions in the laboratory.

A few attempts have been made to study the self-selection of diets under controlled conditions. Animals were given a choice of a number of foods which were present either in their natural habitat or which on theoretical grounds might play a part in their nutrition. Most of these experiments were undertaken in agricultural stations with the hope of finding a cheap and simple method of feeding animals. Evvard (1915) gave pigs a free choice of whole corn grain, meat meal (containing 60 per cent protein), whole oats, linseed oil meal, wheat middlings, charcoal, limestone, salt, and water. The animals showed remarkably rapid growth. One animal was the largest pig of its age ever raised at the Iowa Experimental Station. He also found that young pigs, given free access to salt, showed better growth than pigs which received varying amounts of salt in their regular diet. With Dox (quoted by Evvard, 1915), he showed that a free choice of calcium carbonate also produced increased growth in pigs. Osborne and Mendel (1918) gave rats a choice of two diets, one with a superior, and one with an inferior mixture. A number of such pairs of diets were used, some with high and low protein content, some with

proteins poor and rich in amino-acid content, etc. Ultimately, the rats always chose the superior mixture and showed normal or nearly normal growth. By similar but more extensive experiments on rats and mice, Mitchell and Mendel (1921) confirmed these results. Kon (1931) reported that, given a choice of sucrose, casein, and a salt mixture offered separately, rats did not thrive as well as when these substances were offered in a composite mixture. Pearl and Fairchild (1921) reported that, given a choice of a large variety of natural foods, chickens showed better growth than was obtained under a system of man-controlled mass feeding. Recently Dove (1935) also reported that chickens were able to make advantageous selections from a variety of natural foods. According to him, this ability varied from animal to animal and was dependent on genetic factors. Davis (1928) found that three young children, given a choice of a large number of foods, grew normally and became more healthy. Sweet (1936) found that children made better dietary selections on the basis of appetite than when guided by their parents.

These experiments had in common several shortcomings due to the use of natural foods or synthetic food mixtures. It was believed by most of the workers that appetite could be a guide only when foods were presented in their natural form. However, because of the complexity of the ingredients in natural foods, it was never possible to determine whether an animal was attracted to a single component or to the combination of substances.

These difficulties were overcome to a great extent in self-selection experiments in which purified or nearly purified substances were used (Richter, Holt, Barelare, 1937). The possibility that rats could make selections from purified substances was suggested by the results of experiments on the changes in mineral appetite of adrenalectomized and parathyroidectomized rats. It is well known that adrenalectomized rats die within 10 to 15 days after the operation, apparently because of the excessive loss of salt. It was found that when given free access to salt solutions, adrenalectomized rats voluntarily took a sufficient amount to keep themselves alive and free from symptoms of insufficiency (Richter, 1936). In some animals, the salt intake increased fifteen-fold after adrenalectomy. Parathyroidectomized rats which ordinarily lose weight, show symptoms of tetany, and eventually die, took large amounts of solutions of calcium lactate, 2.4 per cent. Consequently they remained free of tetany and grew normally. The calcium lactate intake was increased twelve fold in one animal after parathyroidectomy. Parathyroid implants to the anterior chamber of the eye caused the calcium appetite to return to its normal level (Richter and Eckert, 1937).

It was decided then that if the deficient rats could select these two minerals to such great advantage, it might be worth while to determine

whether normal rats could make wise selections of other minerals as well as of protein, carbohydrate, fat, and vitamins. The ideal method of determining this would have been to give the rats a choice of all substances which are known to have anything to do with nutrition; however, this would be impractical at this point.

In order to simplify the situation, it was decided to restrict the selections to one representative of most of the substances known to play an important part in the nutrition of the rat, that is, protein, carbohydrate, fat; the minerals, sodium, calcium, phosphorus, potassium; vitamins A, B, D, and E. It has been reported that the rat can produce its own vitamin C (Parsons and Hutton, 1924); therefore, this substance was not offered.

The immediate problem was to select the representative of each group. The fact that this experiment depended entirely upon appetite suggested that the rats themselves should select the representatives. Obviously, this was better than making a priori selections on the basis of our theoretical knowledge of the nutritional value of the foodstuffs. A series of well known fats, carbohydrates, and proteins were offered singly and with no other substance except water. Under these conditions, the survival time was taken as a measure of the nutritional value of the food. The selection of the mineral solutions and their concentrations was also left to the rat. Rats kept on the McCollum diet were given a choice of water and a mineral solution. The ingestion of a small amount of the solution each day was taken to indicate that the mineral was needed and should be included in the multiple food choice experiments. A similar system was used in selecting the substances containing the vitamins, except that the choice was smaller.

METHODS. The rats were kept separately in cages consisting of a revolving drum, a cyclometer, and a living compartment containing food and fluid receptacles. In the preliminary single food choice experiments, the living compartments contained one bottle for water and another bottle or cup for the food to be tested. In the multiple choice experiments, the cages were made large enough to contain 3 cups for solids and 8 bottles for fluids.

The rats were placed in the cages at an average age of 49.5 days and kept on the McCollum diet for 10 days or more before being changed to one of the experimental diets. The McCollum diet contained 725 grams of graham flour, 100 grams of casein, 100 grams of skimmed milk powder, 50 grams of butter, 10 grams of sodium chloride, and 15 grams of calcium carbonate. Lettuce (10 grams) was given weekly.

Daily records were made of activity, intake of solids and fluids, and of vaginal smears; weekly readings were made of body weight. The bottles were cleaned and refilled twice weekly. The animals were kept under constant observation for the appearance of any deficiency symptoms.

The animals were autopsied; the endocrine glands were removed and preserved for histological study.

Seventy-two animals were used in the preliminary single food experiments; 8 animals were used in the multiple food choice experiments. Since these records were obtained, observations have been made on 40 additional animals with essentially the same results.

RESULTS. *Preliminary experiments with choice of single foodstuffs, fat, carbohydrate, and protein. Fat.* Six fats were tested: olive oil, lard, cod liver oil, wheat germ oil, perilla oil, and peanut oil. Groups of 4 animals were kept on each of these fats plus water. Records were made of activity, body weight, and solid and fluid intake.

For the present purposes, only the survival time will be reported. The results are summarized in figure 1. The rats lived longest on olive oil, averaging 46.5 days; next longest on lard, 27.5 days; and shortest on perilla oil, 11 days. Olive oil was thus selected for the multiple food choice experiments.

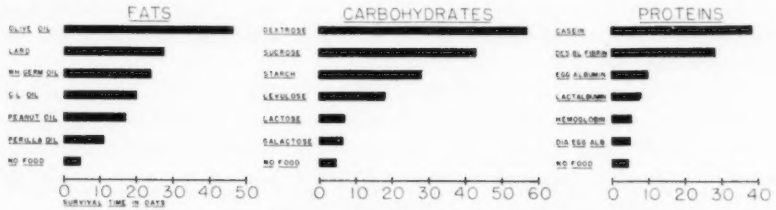


Fig. 1. Survival times of rats on single choice experiments

*Carbohydrate.* Six carbohydrates were tested: dextrose, sucrose, starch levulose, lactose, and galactose. The animals lived longest on dextrose, 57 days, next longest on sucrose, 43 days, and shortest on galactose, 6.5 days. Sucrose, rather than dextrose, was chosen for the multiple choice experiments, because it had already been used in a number of our experiments and because animals lived nearly as long on it as on dextrose.

*Protein.* Six proteins were tested: casein (autoclaved and purified), desiccated blood fibrin, egg albumin, lactalbumin, hemoglobin, and dialyzed egg albumin. The animals lived longest on casein, 38.5 days, next longest on desiccated blood fibrin, 28.5 days, and shortest on dialyzed egg albumin, 5.0 days. Casein was selected for the multiple food choice experiments.

These survival times must be regarded as only rough estimates to serve the present purposes. With records on a larger number of animals, data obtained from this type of experiment should help to throw more light on the fundamental nutritional differences between these various substances.

*Minerals: Sodium.* From the afore-mentioned adrenal experiments it was known that normal as well as adrenalectomized rats show a definite

appetite for sodium chloride offered either in 1 per cent or 3 per cent solutions. Due to the fact that often the 1 per cent solution was not concentrated enough to be distinguished from tap water, the 3 per cent solution was used in the multiple choice experiments.

*Calcium.* It was observed in the parathyroid experiments that normal rats show an appetite for calcium lactate solution and consequently will drink a small amount from day to day. A 2.4 per cent solution was used in the following experiments.

*Phosphorus.* The rats showed a definite appetite for phosphorus in the form of dibasic sodium phosphate. The concentration easily distinguished from water was 8 per cent.

*Potassium.* The preliminary experiments indicated that rats would take potassium chloride in concentrations varying from 1 to 2 per cent. Solutions of 1 per cent were used in the present experiments.

*Vitamins: Cod liver oil.* It was found that rats would take a small amount of cod liver oil each day when it was offered in a container separate from the McCollum food. Due to the fact that vitamin D could not be obtained separately from vitamin A, cod liver oil had to be used to test the appetites for both.

*Vitamin B complex.* At the time these experiments were started, dried baker's and brewer's yeast were the only practical sources of vitamin B. It was found that, given either separately or with the McCollum diet, yeast was readily taken by the rats. Dried baker's yeast was chosen for these experiments because of its powdered form.

*Vitamin E.* The only practical available source of vitamin E was wheat germ oil. The rats drank it quite freely when it was offered either separately or with the McCollum diet.

Thus, in summary, the following substances were used: olive oil, casein, sucrose, sodium chloride, 3 per cent, calcium lactate, 2.4 per cent, dibasic sodium phosphate, 8 per cent, potassium chloride, 1 per cent, yeast, cod liver oil, wheat germ oil, and water. The casein, yeast, and sucrose were offered in the food cups; the solutions were offered in the graduated, inverted bottles.

*Multiple food choice experiments: Effect on growth.* The growth curve for 8 females is presented in figure 2. For purposes of comparison, growth curves are shown also for 19 females on the McCollum diet, and for 50 females kept on a diet of table scraps in the Wistar Institute (Donaldson, 1915). After an initial slight reduction in weight, probably produced by the change from the McCollum diet, the growth of animals on the self-selection diet paralleled almost exactly that of the animals on the McCollum diet. On both of these diets, growth was far better than on the Wistar diet.

*Reproduction.* The 4- to 5-day oestrous cycles, studied by the vaginal

smear method, showed a very striking regularity. They were considerably more regular than on the McCollum diet.

The rats mated, conceived, gave birth to normal litters, and nursed their young quite as well as on any of the synthetic diets. A detailed study of the changes in appetite during pregnancy and lactation on the self-selection diet has been made (Richter and Barelare, 1938).

*Activity.* The average daily activity of the 8 animals on the self-selection diet was essentially the same as that of 19 animals on the McCollum diet. See figure 3. The self-selection curve is slightly lower, but when the great individual variations of animals on both diets are taken into account, this difference is negligible.

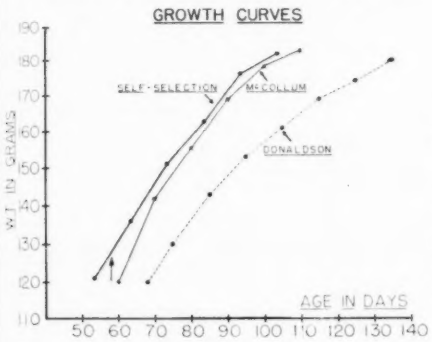


Fig. 2

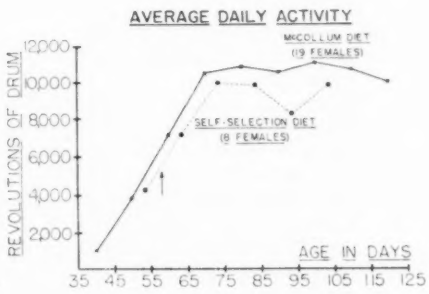


Fig. 3

**Fig. 2.** Growth curves of 8 female rats on self-selection diet, 19 animals on McCollum diet, and 50 animals on the table scrap diet at the Wistar Institute. The arrow at 59 days indicates the average age at which the experimental rats were changed from the McCollum to the self-selection diet.

**Fig. 3.** Curves showing average daily activity of 8 female rats on self-selection diet and 19 female rats on McCollum diet.

*Average daily intake of the various solids and fluids.* The average daily intakes recorded for the 8 animals are presented in table 1. They are based on a 10 day period after the animals had been on the self-selection diet for at least 40 days and had shown normal growth and regular oestrous cycles. The average age of the animals was 100 days. The total solids (including oils) ingested weighed 8.91 grams, which is 36.4 per cent less than the 14.0 grams average for animals of the same age on the McCollum diet. The total fluids imbibed amounted to 22.6 cc. which is about the same as the average water intake of animals on the McCollum diet.

*Total calories and percentage of fat, carbohydrate, and protein.* The average daily total caloric intake on the self-selection diet was 46.3 calories. See table 1. This is 18.7 per cent less than the average total caloric intake of 56.0 calories of animals of the same age on the McCollum diet.

Fat contributed 64.0 per cent, protein 16.0 per cent, and carbohydrate 20.0 per cent of the total calories as compared to 14.1 per cent, 26.7 per cent, 59.2 per cent respectively on the McCollum diet (table 2). Thus it is seen that the animals on the self-selection diet ingested far larger proportions of fat and less of protein and carbohydrate. This explains in part the smaller bulk of the self-selection diet.

*Daily mineral intake.* The mineral content of the self-selection diet is shown in table 3. The average daily intake of sodium was 0.090 gram

TABLE 1

*Table showing average daily intake of solids, fluids, and calories on the self-selection and McCollum diets*

| | SOLIDS | CALORIES | FLUIDS |
|---|---|---|---|
| | *grams* | | *cc.* |
| Self-selection: | | | |
| Casein | 1.30 | 6.7 | |
| Yeast | 1.40 | 1.5 | |
| Sugar | 2.30 | 8.5 | |
| Olive oil | 1.70 | 14.6 | |
| Cod liver oil | 0.14 | 1.2 | |
| Wheat germ oil | 1.60 | 13.8 | |
| NaCl (3 per cent) | 0.04 | | 1.3 |
| KCl (1 per cent) | 0.03 | | 2.5 |
| $Na_2HPO_4$ (8 per cent) | 0.23 | | 2.9 |
| Ca lactate (2.4 per cent) | 0.17 | | 7.2 |
| Water | | | 8.7 |
| Total | 8.91 | 46.3 | 22.6 |
| McCollum | 14.00 | 56.0 | 23.0 |

TABLE 2

*Table showing caloric percentages of fat, protein, and carbohydrate on the McCollum and self-selection diets*

| DIET | FAT | PROTEIN | CARBOHYDRATE |
|---|---|---|---|
| | *per cent* | *per cent* | *per cent* |
| Self-selection | 64.0 | 16.0 | 20.0 |
| McCollum | 14.1 | 26.7 | 59.2 |

which is slightly higher than the amount (0.066 gram) ingested on the McCollum diet. The average daily calcium intake on the self-selection diet was 0.043 gram which is about one third as much as is taken on the McCollum diet (0.127 gram). The average daily phosphorus intake was approximately the same for both diets, 0.081 as compared to 0.074 gram. The potassium intake on the self-selection diet was lower than on the McCollum diet (0.042 as compared to 0.074 gram).

*Daily vitamin intake. Vitamins A and D.* With an average daily intake of 0.14 cc. of cod liver oil containing 600 I. U. of vitamin A and 85 I. U. of vitamin D per gram, the average daily intake of the rats was 84 I. U. of vitamin A and 12 I. U. of vitamin D. See table 4. The averages were considerably higher than those calculated for the McCollum diet.

TABLE 3

*Table showing daily intake of minerals on the McCollum and self-selection diets*

| | SELF-SELECTION | MCCOLLUM |
|---|---|---|
| | *grams* | *grams* |
| Na from NaCl | 0.015 | |
| Na from $Na_2HPO_4$ | 0.075 | |
| Total sodium | 0.090 | 0.066 |
| Ca from Ca lactate | 0.032 | |
| Ca from yeast | 0.001 | |
| Ca from casein | 0.010 | |
| Total calcium | 0.043 | 0.127 |
| P from $Na_2HPO_4$ | 0.051 | |
| P from yeast | 0.010 | |
| P from casein | 0.020 | |
| Total phosphorus | 0.081 | 0.074 |
| K from KCl | 0.013 | |
| K from yeast | 0.029 | |
| Total potassium | 0.042 | 0.074 |

TABLE 4

*Table showing average daily vitamin intake on the self-selection and McCollum diets*

| | A | B | G | D |
|---|---|---|---|---|
| | *I. U.* | *Sh. U.* | *Sh. U.* | *I. U.* |
| Self-selection | 84 | 31 | 24 | 12 |
| McCollum | 18 | 18 | 17 | 1 |

Since cod liver oil contains both vitamins, it is not possible to draw any definite conclusions regarding the appetite for either A or D. We do know that rats with all the signs of vitamin A deficiency will drink large quantities of cod liver oil, as much as 5 cc. per day, and as a result show an almost immediate recovery. Furthermore, we know that normal rats will ingest small amounts of Carotene (Smaco) daily with considerable constancy.

*Vitamin B complex.* With an average daily intake of 1.4 grams of dried baker's yeast containing 8.6 Sherman units of vitamin $B_1$, and 14 Sherman units of vitamin G per gram, the daily vitamin intake from this source was 12 Sherman units of vitamin $B_1$ and 19 Sherman units of G. With an average daily intake of 1.6 cc. of wheat germ oil containing 12 Sherman units of $B_1$ and 3 Sherman units of G per gram, the daily intake from this source was 19 Sherman units of $B_1$, and 5 Sherman units of G. The totals are given in table 4. These values are only slightly higher than those calculated for the McCollum diet.

*Vitamin E.* We were unable to determine the vitamin E content of wheat germ oil intake. Since the rats showed such a great craving for fat, and since their appetite for wheat germ oil is almost as great as for olive oil, it may be assumed that a large part, if not all of the appetite for wheat germ oil was for fat, rather than for any of its other components.

*Vitamin C.* It was mentioned above that rats apparently are able to synthesize their vitamin C from their food and that for this reason it was not offered for choice. It is interesting to report, however, that given free access to ascorbic acid (Cebione, Merck), rats will drink small amounts daily with considerable regularity. It is possible, of course, that they may be attracted to the acid rather than to the vitamin content.

*Individual differences.* The selections made by the 8 animals were strikingly similar, except for sucrose and olive oil. Two animals chose larger amounts of sucrose and smaller amounts of olive oil ("sugar burners"); six chose more fat than carbohydrate ("oil burners").

The "sugar burners" usually become "oil burners" after several months. No instances have been recorded in normal animals of a change in the opposite direction. In general, there appears to be a tendency for animals to use more fat as they grow older. This explains the higher carbohydrate intake of younger animals in our preliminary report.

Discussion. The results of these experiments demonstrate the ability of rats to select from purified substances a diet which is conducive to normal growth and reproduction. The difficulties present in some of the previous self-selection studies thus must have been due to the complex nature of the natural foods or of the food mixtures offered for choice.

Despite the fact that ingredients of natural foods rarely appear separately, animals apparently are able to recognize them in their isolated forms. There must be some mechanism by which animals are able to recognize not only sugar and salt, but other minerals, vitamins, proteins, and fats. The existence of special appetites for these substances was suggested by Turro (1910–1911) on the basis of theoretical speculations regarding the underlying mechanisms of appetite and hunger.

How the animals are able to make such advantageous dietary selections is not known. There are those who believe that all self-selection choices

are based on experience, or a trial and error process. This might apply in vitamin B deficient rats in which the beneficial results of yeast (vitamin B) ingestion appear almost at once, in fact, within less than an hour (Harris, et al., 1933). But certainly this does not apply to normal animals in which the effects of eating any substance, except possibly a strong poison, do not appear usually for many hours or even days. The fact that all of the rats made essentially the same choices, and without any apparent experimentation, indicates that some other mechanism must be involved. The consistent selections made by different species also suggests another explanation. To explain the specific food choices, Turro (1910–1911) postulated a "trophic center" in the basal ganglia, but there is little evidence to substantiate such a theory. It would seem more likely that nutritive deficits produce physicochemical changes throughout the entire body, including the taste mechanisms in the mouth, and that these changes may entirely alter the taste for different substances. In response to these changes an animal may be stimulated to seek certain substances in much the same way as dehydrated animals are stimulated by a dry throat to seek water. On the basis of theoretical considerations on appetite and hunger, Mursell (1925) arrived at very similar conclusions. Thus he states, "the best hypothesis covering these facts is that of certain positive chemotropisms which operate to set up cravings for specific substances."

This problem will have to be solved experimentally by studying the effects produced on the various appetites by division of the taste nerves, or destruction of the taste buds on the tongue and in the mouth.

It may be pointed out that in this method we have for the first time a real tool for the study of gustatory localization in the cortex and brain stem. Experiments are now in progress on the effects produced on dietary selections by removal of different parts of the cortex.

These experiments have several shortcomings. In the first place, the diet did not contain some of the most important minerals: iron, manganese, magnesium, copper, and cobalt. It is undoubtedly true that the lack of these would have eventually produced some defect. We do know from preliminary experiments using the technique described in the present paper that rats have an appetite for all of these minerals. In addition, the diet did not contain vitamin C.

Another shortcoming of the experiments is the use of yeast which contains at least 3 important substances with different functions, vitamin $B_1$, riboflavin, and nicotinic acid, in addition to large amounts of protein and carbohydrate.

## SUMMARY

1. A study was made of the ability of rats to select their diet from a number of purified foods.

2. The assortment offered in separate containers to the animals included one representative each of the more important nutritional substances: olive oil, casein, sucrose, cod liver oil, wheat germ oil, yeast, sodium chloride, calcium lactate, sodium phosphate, and potassium chloride.

3. The 8 animals used in these experiments made selections conducive to normal growth and reproduction. They grew as rapidly and were as active as animals on the standard McCollum diet and showed strikingly regular oestrous cycles. They mated, gave birth to normal sized litters, and nursed them until the time of weaning.

4. Despite the same rate of growth, the average daily caloric intake was 18.7 per cent less than on the McCollum diet, 46.3 calories as compared to 56.0 calories.

5. The weight of the solids (including oils) was 36.4 per cent less, 8.91 grams as compared to 14.0 grams on the McCollum diet.

6. The results showed that the rat has a special appetite not only for salt and sugar, but also for protein, carbohydrate, sodium, calcium, phosphorus, potassium, and the vitamins.

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# AUTONOMIC CONTROL OF THE RETRACTOR PENIS IN THE CAT

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Received for publication February 3, 1938

Several studies have been made of smooth muscle having a single autonomic nerve supply, as in the nictitating membrane and pilomotors. The retractor penis represents another instance of readily accessible smooth muscle. It has a dual nerve supply (Langley and Anderson, 1895): derived from the sympathetic almost exclusively by way of the abdominal sympathetic chain; and the parasympathetic *via* the visceral (pelvic) branches of the first two or three sacral nerves. It was considered important to compare this muscle with those simpler systems previously studied which have a single nerve supply. In addition, it was important to determine how this more complex autonomic neuro-effector system stands in relation to the current theories of chemical mediation of nerve impulses.

METHODS. Cats were used for all experiments. Anesthesia was produced by dial (Ciba, 0.55 cc. to 0.80 cc. per kgm.), given intraperitoneally. The animals were placed supine with the hind legs strongly flexed on the pelvis. Isotonic recording from the tip of the intact penis, using a serrefine for attachment, was uniform practice. Two types of control were used. In some instances a second lever was used to record from the skin or muscles of the perineum in order to detect any changes taking place there which might influence the penis. Changes were most frequently absent, but when present were small in degree and not correlated with movements of the penis brought about by the retractor. In other instances a stout needle was driven through the base of the penis, and with the handle of the needle firmly fixed in a clamp movement at the base was prevented. Under these conditions any change in the length of the organ is brought about by the retractor pulling the tip toward the base. Always a short incision, dorsal and ventral, was made in the prepuce the better to expose the organ.

The preganglionic sympathetic supply was stimulated with shielded silver electrodes (inter-electrodal distance 5 mm.) placed under both sympathetic chains at $L_7$, the chains being cut at $L_1$. The parasym-

pathetic supply was stimulated by placing shielded silver electrodes (inter-electrodal distance 15 mm.) under the cauda equina at the level of the iliac crests. In these conditions the cord was cut at $L_1$, and curare with artificial respiration was necessary. Sections of the cord and sympathetic chains were practiced to insure the elimination of reflex effects and to guarantee that all the responses obtained were peripheral.

Injections of drugs were made into an external jugular vein. Stimulation was provided by a condenser set connected with a mechanical interrupter. When stimulation against a background of activity was desired, the background was established by means of a metronome interrupting the primary circuit of a Harvard induction coil at slow frequencies (1 to 4 per sec.). Stimuli in all cases were supramaximal to rule out any spatial summation.

Results. A. *Temporal summation with stimulation of the lumbar sympathetics* (l.a.s.). Cats with lumbar cord pithed and l.a.s. cut at $L_1$ were used. It was deemed advisable to investigate the curves correlating the response to graduated frequencies of stimulation, for, as was pointed out by Rosenblueth (1932) these curves are usually rectangular hyperbolae. Figure 3A shows a typical curve. All such response-frequency curves were shown to be rectangular hyperbolae by plotting frequency ($F$) against frequency over response ($F/R$), whereupon straight lines were obtained. In figure 1 a typical response to l.a.s. stimulation is shown at $A$. It is clearly pure contraction, with no subsequent inhibition.

B. *Responses to graduated doses of adrenalin.* A typical response of the retractor penis to adrenalin is shown in figure 1B. For these observations the l.a.s. were cut at $L_1$ and the lumbar cord was pithed. The response-dose curves obtained were characteristically S-shaped (fig. 3B). The initial "tail", however, largely disappeared if the series was taken after cocaine (8 mgm. per kgm.; fig. 3B).

C. *Effect of cocaine on responses to stimulation of the l.a.s. and adrenalin.* In order to investigate this effect a given dose of adrenalin intravenously and a definite strength of stimulus to the l.a.s. were selected. After separate application of these two stimuli, 8 mgm. of cocaine per kgm. were injected intravenously. The adrenalin and the l.a.s. stimulation were then repeated. Both responses were increased in magnitude and duration by the cocaine (fig. 1).

D. *Temporal summation to parasympathetic (cauda equina) stimulation.* The l.a.s. chains were removed from $L_1$ to $S_2$ 10 to 14 days before the acute experiments. Stimuli of varying frequency were applied to the cauda; curare and artificial respiration were necessary to eliminate somatic motor activity. The response-frequency curves obtained proved to be rectangular hyperbolae, verified in the usual manner (fig. 4A, B). The responses showed typically a preliminary sudden rise of short duration, then a

marked relaxation and a very gradual return to the base line (fig. 2A). In a few instances the initial rise was absent, the curve representing a pure relaxation (fig. 2B). The return to normal in the second case was quite as gradual as in the first. Stimulation of the cauda was found to be little, if any, potentiated by eserine. One must note, in addition, that the initial rise was frequently present after chronic lumbar sympathectomy.

E. *Interaction of sympathetic and parasympathetic effects.* Only qualita-

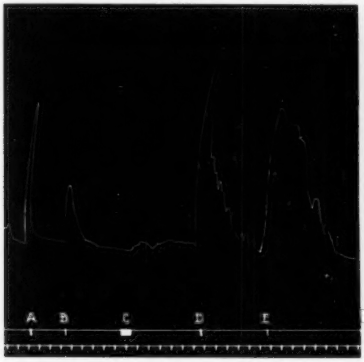


Fig. 1

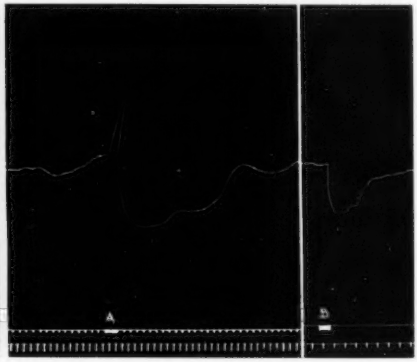


Fig. 2

Fig. 1. Isotonic contractions of a cat's retractor penis to stimulation of the lower abdominal sympathetics at $L_2$ and to injected adrenalin, before and after cocaine. Lumbar cord pithed, lower abdominal sympathetics cut at $L_1$. Dial anesthesia. Time recorded at 30-sec. intervals.

A. Lower abdominal sympathetics stimulated at a frequency of 10 supramaximal shocks per sec.

B. Adrenalin, 50γ, injected into a jugular vein.

C. Cocaine, 20 mgm., injected into a jugular vein.

D. A repeated.

E. B repeated.

Fig. 2. Isotonic relaxations of a cat's retractor penis on stimulation of the cauda equina. Dial, curare, artificial respiration. Time recorded at 5-sec. intervals.

A. Diphasic response to 4 supramaximal shocks per sec.

B. Monophasic response to 5 supramaximal shocks per sec.

tive studies were carried out. In one group of cats (cord cut at $L_1$, l.a.s. cut at $L_1$) the cauda was stimulated (curare and artificial respiration) for a definite period of time. During this continuous stimulation the l.a.s. was stimulated for a short interval. Regardless of the frequency used on the cauda, responses were always obtained by stimulating the l.a.s. at any frequency which would elicit a response if applied alone. Even when the frequencies delivered to the cauda were raised to 90 per sec., responses were obtained from concurrent l.a.s. stimulation (fig. 6B).

The reverse of this experiment, a short period of cauda stimulation, superimposed on a longer period of l.a.s. stimulation, also yielded responses through all ranges (fig. 6a).

Acute section of the l.a.s. is followed by a relaxation of the retractor. Hence, when it was desired to stimulate the cauda and superimpose l.a.s. excitation it became necessary to establish an initial tone of the retractor by slow (1 to 4 per sec.) l.a.s. stimulation. It was on this background that a period of cauda relaxation was produced with a short period of stronger l.a.s. stimulation superimposed (fig. 6b). Of course, if this were not done, cauda stimulation would show nothing but a straight line, since early after section of the l.a.s. in these acute experiments the retractor is fully relaxed (Langley and Anderson, 1895).

F. *Acetylcholine effects.* In order to study the action of acetylcholine (a-ch.) on the retractor penis it was necessary to prepare animals with chronic lumbar sympathectomies. The l.a.s. were aseptically removed from $L_1$ to $S_2$. This prevented the possible activation of the ganglia by large doses of a-ch. (up to 0.4 mgm.). After 10 to 14 days the experiments were performed. Both adrenals were excluded from the circulation, obviating any release of adrenine by the medulla in response to a-ch. A relaxation was always produced by a-ch., frequently preceded by a small contraction of short duration (fig. 5A). The major relaxation was potentiated by eserine (0.5 mgm. per kgm.) (fig. 5C) and was completely blocked by atropine (fig. 5E). The preliminary rise, when present, persisted after atropine (fig. 5E). It was, however, blocked by curare or by removing the remainder of the l.a.s. below $S_2$ on the front of the sacrum.

Discussion. A. *Effects of l.a.s. stimulation and administration of adrenalin.* The results point toward chemical mediation by sympathin. Adrenalin reproduces l.a.s. stimulation (fig. 1). Both stimulation of l.a.s. and adrenalin are potentiated by cocaine (fig. 1).

The S-shaped curves obtained with adrenalin before cocaine (fig. 3B) may be due to the fact that some of the injected adrenalin must of necessity be lost in the blood and will not take part in producing the response. Cocaine tends to obliterate the "tail" of the S-shaped dose-response curve, probably by rendering access to the effector cells easier (Rosenblueth and Schlossberg, 1931; Cannon and Rosenblueth, 1937).

B. *Parasympathetic stimulation and acetylcholine.* Here also the response-frequency curves were found to be rectangular hyperbolae. As regards the nature of the parasympathetic mediator, the lack of influence of atropine on the relaxation elicited by the nerve impulses (Langley and Anderson, 1895; Henderson and Roepke, 1937) is not discriminating. In the stomach, similarly, atropine abolishes the responses to a-ch. while not paralyzing those to vagal stimulation, yet a-ch. is the chemical mediator of the nerve impulses (Dale and Feldberg, 1934).

Effects of stimulation of the cauda (fig. 2A) are duplicated by a-ch. (fig. 5A). The action of a-ch. is potentiated by eserine (fig. 5), but the results of cauda stimulation are little changed. It is possible that this muscle contains little choline esterase. Further evidence of this is the long duration of parasympathetic effects (up to 150 seconds). When a-ch. is injected it is easily antagonized by atropine (fig. 5E), while a-ch. released intracellularly by nerve stimulation is relatively inaccessible to injected atropine and thus may continue to be effective (Dale and Gaddum, 1930). In fact, occasionally after eserine, if doses of a-ch. of the order of 0.01 mgm. are injected, the retractor relaxes completely and does not recover during the time available for an ordinary acute experiment.

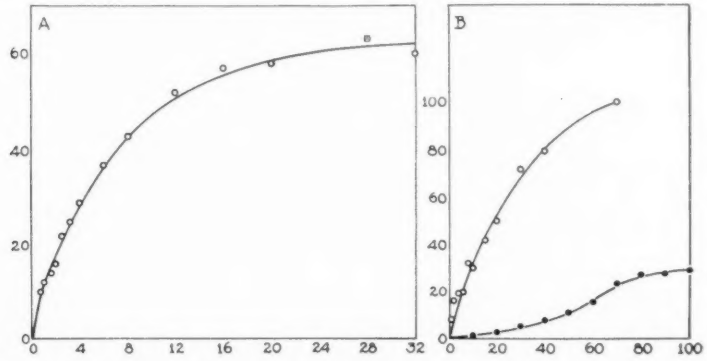


Fig. 3A. Frequency-response curve for cat's retractor penis. Abscissae: frequencies per sec. of stimulation of the lower abdominal sympathetic chains at $L_7$ with supramaximal shocks. Ordinates: responses in millimeters in the record.

B. Dose-response curves to adrenalin for cat's retractor penis. Abscissae: doses of adrenalin in $\gamma$. Ordinates: responses in millimeters in the record. Dots: before cocaine. Circles: after cocaine (8 mgm. per kgm.).

The initial contraction with a-ch. is shown to be a nicotine ganglion effect by the fact that after eserine and curare small doses (1$\gamma$ to 0.01 mgm.) are sufficient to show a marked potentiation of the muscarine effect, while the nicotine effect at the ganglion in this a-ch. dose range is completely blocked. Large doses of a-ch. (0.4 mgm.) after atropine and curare will be sufficient to bring out the nicotine effect without the relaxation produced by muscarin effects of a-ch. Curare probably acts by raising the threshold of ganglia for a-ch. Hence small doses of a-ch. (0.01 mgm.) are blocked, while large doses (0.4 mgm.) are above the raised threshold, and thus bring out the ganglion effect and cause shortening of the retractor. Further evidence has been obtained that the preliminary contraction in a diphasic response to a-ch. (fig. 5A) is a nicotine ganglion effect from the fact that removing or destroying the ganglion at $S_3$, not

removed at the preliminary chronic operation, will also prevent the appearance of this initial contraction. Langley and Anderson (1895) showed that the fibers from $S_3$ in the cat frequently supply the retractor and this ganglion can not be readily reached at the time of the chronic operation.

C. *Simultaneous stimulation of sympathetic and parasympathetic nerves.* Bozler (1936) likens the action of mediators on blood vessels to Sherrington's c.i.s. and c.e.s., where the action of c.i.s. is not on the lower moto-

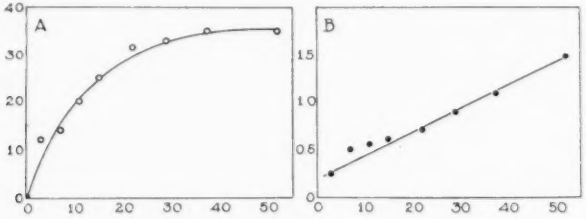


Fig. 4A. Frequency-response curve of a cat's retractor penis. Abscissae: frequencies per sec. of stimulation of the cauda equina with supramaximal shocks. Ordinates: responses in millimeters in the record.

B. Abscissae: frequencies per sec. of stimulation applied to cauda equina (as in fig. 4A). Ordinates: ratio of frequency to response ($F/R$). The straight line indicates that the curve in figure 4A is a rectangular hyperbola.

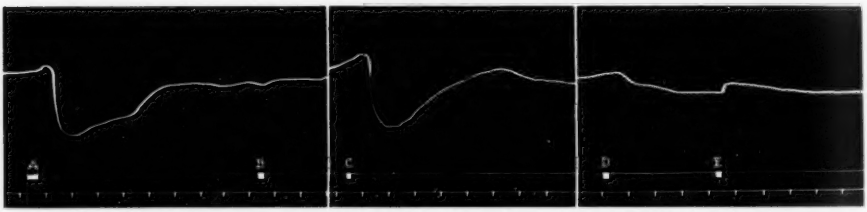


Fig. 5. Isotonic recording of a cat's retractor penis. Responses before and after eserine, and after atrôpine. Lower abdominal sympathetics removed aseptically 10 days before this acute experiment. Adrenals excluded acutely.

A. Response to 400$\gamma$ acetylcholine.
B. Eserine, 2 mgm.
C. Acetylcholine, 10$\gamma$ (potentiation effect).
D. Atropine, 3 mgm.
E. Acetylcholine, 400$\gamma$.

neurone to the effector but serves only to neutralize c.e.s. On the other hand, Rosenblueth and Simeone (1934) have shown that for the pacemaker of the heart, in the case of the vagus and accelerators, the effect of simultaneous excitation is the resultant of the two influences. Two effects occur independently. The retractor penis in the cat, even with maximal (90 per second) stimulation of the cauda, still responds to all ranges of sympathetic l.a.s. stimulation (fig. 6b). If the mediator re-

leased by cauda stimulation acted to neutralize that produced by l.a.s. stimulation, at this high frequency all sympathetic activity should be blocked or at least reduced. Such is not the case; cauda stimulation does not inhibit or diminish l.a.s. responses. In some instances l.a.s. responses are slightly increased against a background of cauda activity as compared to the response obtained by l.a.s. stimulation alone. It is also true that cauda stimulation will cause response against all ranges of l.a.s. activ-

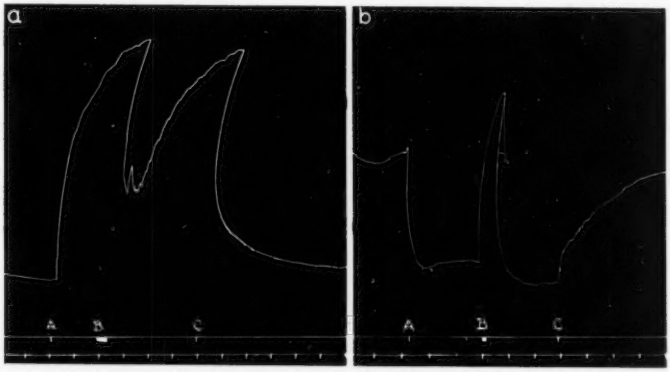


Fig. 6a. Isotonic recording of a cat's retractor penis. Dial, curare, artificial respiration. Cord sectioned at $L_1$, abdominal sympathetic chains sectioned at $L_1$. Time recorded at 30-sec. intervals.

A. Beginning of continuous stimulation of l.a.s. with supramaximal shocks at 8 per sec. Coil at 9.

B. Stimulation of cauda equina with supramaximal shocks at 3 per sec.

C. End of continuous stimulation of l.a.s.

Fig. 6b. Isotonic recording of a cat's retractor penis. Dial, curare, artificial respiration. Cord sectioned at $L_1$, abdominal sympathetic chains sectioned at $L_1$. Time recorded at 30-sec. intervals. Background of sympathetic activity provided by supramaximal shocks applied to l.a.s. at $L_7$ at a frequency of 4 per sec. because of complete relaxation following acute section of l.a.s. and cord.

A. Beginning of continuous stimulation of cauda equina with 90 supramaximal shocks per sec. Coil at 0.

B. Stimulation of l.a.s. at 52 per sec. with supramaximal shocks.

C. End of continuous stimulation of cauda.

ity (fig. 6a). The effects, therefore, occur independently. Although a smooth muscle, the retractor penis may be compared to the cardiac pacemaker in its behavior toward the two mediators acting upon it.

## SUMMARY

The temporal summation of the responses of the cat's retractor penis when stimuli are applied to either the sympathetic or parasympathetic nerves obeys a hyperbolic law, similar to that which occurs in other simpler autonomic effectors (figs. 3 and 4).

Evidence is presented that the mediator of the sympathetic nerve impulses is sympathin, i.e., that these nerve fibers are adrenergic.

Similar evidence suggests that the parasympathetic nerve supply is cholinergic.

The two mediators act independently on the effector (fig. 6); one does not prevent the action of the other. The effect of simultaneous stimulation is the resultant of the two opposite influences.

The smooth muscle of the retractor penis probably contains little cholinesterase.

It is my wish, at this time, to express to Dr. A. Rosenblueth my appreciation of his most valuable advice and encouragement. To Mr. T. Barnett my thanks are due for his assistance during the sterile surgical operations.

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# THE BEHAVIOR OF THE EMBRYONIC HEART IN SOLUTIONS OF OUABAIN

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Received for publication February 4, 1938

The embryonic chick heart in the tubular stage offers a relatively simple system for the study of drugs of the digitalis group. At 48 hours the cardinal functional properties of heart muscle are well developed and despite the absence of both a conduction system and the influence of nerves a definite sequence of events occurs with each heart cycle. The venous or sino-atrial end of the heart acts as a pace-maker (1, 2), and the ordered sequence of contraction as the impulse sweeps from venous to arterial end of the heart can be followed with ease under the microscope.

Pickering (3), Hall (4) and Neter and Witebsky (5) have made observations on the embryonic heart treated with digitalis, but the method employed placed definite limitations on the ability to vary conditions and to observe effects. These experimenters studied the heart in situ.

METHODS. Isolated hearts of approximately 48 hours of incubation age were used. With a simplified tissue culture technique it was possible to vary conditions at will and to observe effects under the microscope. Three procedures were followed. In the first a series of drops, some of Tyrode solution and some of ouabain-Tyrode solution were arranged on a glass slide which was placed inside a covered Petri dish containing moist cotton. Under constant temperature (38–39°C.) an isolated heart was then transferred from one drop to another within this moist chamber in order to study the effect of the ouabain. While critical examination was difficult, due to free movement of the heart in the liquid medium, we found this method especially suitable for studying changes in rate.

In the second procedure an isolated heart was transferred to a small amount of chicken blood plasma which had been placed on the inside wall of a small bottle. After the plasma had clotted the bottle was placed in an upright position and the solution to be tested was pipetted into the bottom of the bottle without bringing it in contact with the plasma. The bottle, tightly stoppered, was then placed in an incubator where time could be allowed for the entire preparation to reach a uniform temperature before bringing the solution in contact with the plasma and heart. This method

was most suitable for determining whether or not hearts stopped by the action of ouabain could be revived by washing.

The third procedure which was more suited for quantitative studies is the one we used in most of the work. Ouabain-Tyrode solution of twice the desired ouabain concentration was diluted with an equal volume of blood plasma. A small drop of this mixture was then transferred by means of a vaselined platinum loop to the surface of a coverslip, the isolated heart submersed in the drop, covered with a hollow ground slide and sealed. When timing of events was desired a stop-watch was started as the heart was submersed. The clot which forms in such a preparation is firm enough to prevent any swimming movement of the heart without interfering appreciably with its contractile activity.

Observations. The first group of experiments involved the use of isolated hearts in ouabain diluted to 1:300,000 at a temperature of 38–39°C. When observed under the microscope a quite constant train of events occurs as follows. There is an initial slight acceleration in the rate of beat which precedes any indication of disturbance in rhythm. This was unexpected since both Pickering (3) and Neter and Witebsky (5) had reported a slowing of the heart when treated with ouabain. Their observations, however, were made upon hearts in ovo after 70 hours of incubation.

Except for the slight change in rate the first noticeable effect is a definite and abrupt block in the region of the atrio-ventricular junction. This disturbance in rhythm is first manifested by occasional dropped beats of the ventricle. The block becomes progressively more severe and soon shows a 2 to 1 sequence. This A-V block is sometimes preceded by a block in the region of the conus which later either moves in a progressive manner toward the atrial end of the heart so as to involve more and more of the ventricle, or, as is more often the case, suddenly jumps to the atrio-ventricular junction region. In the latter case the conus usually follows the rhythm of the ventricle, but occasionally a double block persists so that a sequence such as 4:2:1 is set up in the three parts of the heart.

The degree of block increases still further until finally the ventricle stops completely, the atrial rhythm remaining unchanged. Here again the behavior of the isolated heart seems to differ from that of the heart in ovo as reported by Pickering (3) and Neter and Witebsky (5). In slightly older hearts they found that ouabain stopped the ventricle in systole while in our preparations the ventricles always stopped in diastole. Preceding the cessation of ventricular activity we usually observed a progressive decrease in diameter due to increasingly smaller diastolic excursions, but following the last ventricular systole there is a definite relaxation. These observations have been verified by cinematographic studies.

The conus region sometimes continues to beat for a time after the ventricle has stopped but these contractions are not at all normal. They are slow and of long duration.

The last part of the heart to stop beating is the sino-atrium. It almost invariably continues to beat longer than either the ventricle or the conus. Without apparent change in rate or rhythm the contractions in this portion grow progressively weaker until finally they can no longer be distinguished.

On conducting these experiments we were much interested in the time of appearance of the various effects. In table 1 is listed the time of appearance of atrio-ventricular block and of ventricular stoppage. These experiments were conducted in four separate groups of five each.

The observation was made early that the most constant effect as to time of appearance was the onset of the A-V block. In contrast to this ventricular stoppage was very irregular (cf. table 1), and sino-atrial stoppage

TABLE 1

*Isolated hearts: 1:300,000 ouabain*

(Time in minutes and seconds unless otherwise indicated)

| HEART | A-V-BLOCK* | V-STOP† | HEART | A-V-BLOCK | V-STOP |
|---|---|---|---|---|---|
| 1 | 2:45 | 13 | I | 4:35 | 6:45 |
| 2 | 4:55 | 21 | II | 3:10 | 11:30 |
| 3 | 4:40 | 90 | III | 5:30 | 31 |
| 4 | 3:10 | 29 | IV | 4:15 | 9:10 |
| 5 | 4:30 | 53 | V | 2:40 | 9:30 |
| | Mean 4:00 | | | Mean 4:02 | |
| A | 4:20 | 7 | a | 4:06 | |
| B | 5:30 | 24+ hours | b | 4:23 | |
| C | 3:15 | 24+ hours | c | 3:30 | |
| D | 3:15 | 16− hours | d | 3:45 | |
| E | 4:35 | 24+ hours | e | 3:28 | |
| | Mean 4:11 | | | Mean 3:50 | |

Mean A-V-Block = 4:01. Mean V-Stop = not feasible.

* A-V-Block = atrio-ventricular block.

† V-Stop = ventricular stoppage.

was even more so. The figures have relative value only since the hearts were placed in the warm chamber at 38–39°C. after immersion in the ouabain-Tyrode-plasma mixture at room temperature. (The importance of temperature is discussed below.)

In dilutions of ouabain higher than 1:300,000 the behavior differs. At 1:600,000 the appearance of A-V block is appreciably delayed and is usually preceded by a block in the conus region which progresses through the ventricle toward its junction with the sino-atrium. At 1:1,200,000 the block fails to appear in most cases and the heart beats slower and slower, becomes irregular and finally stops.

The effect of temperature is striking. Dilutions of 1:300,000 which

produced an A-V block in approximately 4 minutes at 38–39°C. required from 6 to 8 minutes at 28 to 29°C. and at 21°C. the average was 14 minutes for three experiments. At a dilution of 1:600,000 the block can be made to appear and disappear at will in the same preparation by merely leaving the heart in the incubator, removing to room temperature, returning it to the incubator, etc.

The next group of experiments was conducted to determine whether or not hearts which had ceased to beat under the influence of ouabain could be revived. In most of the experiments we employed the bottle procedure described above in which the heart was held in position in a drop of clotted plasma. After all activity had ceased the ouabain solution in the bottle was replaced by Tyrode solution. Other hearts were simply submersed in a pool of ouabain-Tyrode mixture until cessation of activity, and then transferred to pure Tyrode. In both methods there was complete revival of all the hearts. Activity began in the sino-atrial portion, passed through a stage resembling partial A-V block, and eventually took on the appearance of a perfectly normal beat. This effect is more striking when we consider that some of the hearts were revived after having been left in the ouabain for two hours after cessation of activity.

Discussion. The term "block" as we have used it in describing these experiments implies that certain impulses arising in the sino-atrium fail to elicit a ventricular response. This is explainable on either of two suppositions: first, either the impulses fail to reach the ventricle, or second, reaching it, they find the ventricle unresponsive. In the first case the block would be due to a depression of conduction, in the second to a decrease in excitability of the ventricle. Several observations which we have made offer strong evidence that the block produced by ouabain in the isolated 48 hour heart is due partly if not wholly to decreased ventricular excitability. *a.* In hearts which have stopped beating under the influence of ouabain strong electrical stimulation fails at first to elicit a response. *b.* After washing with Tyrode solution for a few minutes these same hearts begin to respond to strong electrical and later to weak electrical and mechanical stimuli, but do not beat spontaneously. *c.* With continued washing normal spontaneous activity returns. *d.* Since there is no preferential pathway of conduction in these hearts[1] it might be thought that cutting one-half to two-thirds through the heart in the region of the

[1] Although generally recognized, this fact seems to be based on anatomical rather than physiological evidence. We verified it by subjecting a series of hearts to incisions in the A-V junction region so that all parts of the circumference were cut. In none of these was normal rhythm disrupted. Furthermore we made alternate transverse cuts along the ventricle, one from one side, the next from the opposite side, so that an impulse had to traverse a devious pathway from sino-atrium to conus. Here too no interference with normal rhythm was observed.

A-V junction would render the heart more susceptible to block by ouabain. However hearts treated in this way and then exposed to ouabain continued to beat with a normal rhythm as long as did intact hearts. *e.* If a heart beating normally in Tyrode solution is cut completely across, or ligated in the region of the A-V junction the isolated ventricle continues to beat with a regular rhythm distinctly slower than that of the sino-atrium (1, 2, 6, 7, 8). *f.* Such isolated ventricles, when subjected to the action of ouabain, behave very similarly to the ventricles of entire hearts treated in the same manner. They beat regularly for a time and then suddenly begin to drop occasional beats. The dropped beats become more and more frequent until the contractions finally cease. Furthermore the average time of appearance of the first dropped beats in a series of isolated ventricles corresponds closely to that of the first dropped ventricular beats in a similar series of whole hearts (cf. tables 1 and 2).

TABLE 2

*Isolated ventricle-conus—1:300,000 ouabain*

(Time in minutes and seconds)

| VENTRICLE CONUS | VENTRICULAR IRREGULARITY | VENTRICULAR STOP | VENTRICLE CONUS | VENTRICULAR IRREGULARITY | VENTRICULAR STOP |
|---|---|---|---|---|---|
| 1 | 3:35 | 4:00 | A | 4:05 | 8:00 |
| 2 | 3:20 | 5:30 | B | 4:20 | 8:15 |
| 3 | 2:50 | 6:05 | C | 4:22 | 6:30 |
| 4 | 3:20 | 10:30 | D | 3:15 | 7:30 |
| 5 | 4:05 | 8:40 | E | 6:15 (?) | 10:20 |
| a | 5:20 | 8:40 | I | 2:57 | |
| b | 5:10 | 6:05 | II | 4:20 | |
| c | 4:35 | 9:00 | III | 4:40 | |
| d | 4:45 | 8:45 | IV | 4:14 | |
| e | 3:20 | 10:30 | V | 3:20 | |

Mean V-Irregularity = 4:06. Mean V-Stop (15) = 7:53.

It is clear from these observations that depression of conduction alone cannot be responsible for the block, for ventricles continue to beat when completely separated from the atrial portion. There is also evidence that excitability of the entire heart is lowered for electrical and mechanical stimulation and that this excitability returns when the ouabain is washed out. Whether the first appearance of block is due to depression of conduction in the A-V region, whether it is due to depressed irritability in the ventricular portion, or whether it is brought about through a combination of these two factors, we cannot be certain without further evidence. It is possible that the first dropped beats in the ventricle may be due to failure of the normal impulse to reach the ventricle. By the time the block has reached a 2:1 stage, however, the ventricle is beating slower than it nor-

mally would when completely isolated from the atrial portion, and it seems probable that depressed irritability is a major factor. And the marked similarity in the behavior of the ventricle, whether it be observed in a whole heart preparation or whether it be completely isolated from the atrial portion, makes it seem doubtful that depression of conduction plays any part at all in the block produced by ouabain.

There is much evidence, both clinical and experimental, that the block produced by digitalis in the human heart "is referable, in part at least, to the action of the drug upon the musculature of the ventricle, rendering it less excitable to auricular stimuli" (9). While our experiments tend to support this view as far as the direct action of the drug upon the heart is concerned, its action in an adult heart with a well developed nervous influence and conducting system may be quite different.

## SUMMARY AND CONCLUSIONS

The 48 hour embryonic chick heart exposed to a 1:300,000 dilution of ouabain undergoes A-V block, ventricular stoppage and finally sino-atrial stoppage. The block is most constant as to time of appearance. Higher dilutions or lower temperature greatly delays these effects. These reactions are reversible since a quiescent heart can be revived by washing with Tyrode solution. The cause of block apparently lies primarily in depression of irritability.

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# THE INFLUENCE OF NEMBUTAL, PENTOTHAL, SECONAL, AMYTAL, PHENOBARBITAL, AND CHLOROFORM ON BLOOD SUGAR CONCENTRATION AND CARBOHYDRATE MOBILIZATION

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Received for publication February 7, 1938

During a study of the factors influencing the susceptibility of animals to barbiturate anesthesia, our attention was called to various physiological responses to these compounds (1, 2, 3, 4). This paper presents the results on some carbohydrate changes obtained in rabbits with nembutal, pentothal, seconal, amytal, phenobarbital, and chloroform.[1] The action of phenobarbital persists for more than 24 hours, in some cases as long as 96 hours; the action of the remaining compounds is of short duration, ranging from 2 to 4 hours. Of these, pentothal is the least and amytal the most persistent.

In the course of a study on the duration of the anesthesia produced by the barbiturates, there was found to be no correlation between the susceptibility to the drug and the blood sugar levels, either before the administration of the drug or at the time of deepest anesthesia. Although animals deprived of food for 24 hours showed an increased susceptibility to the anesthetic, this could not be correlated with the blood sugar concentrations (1, 2). Since the lowering of the blood sugar does not explain the increased effects of the drug, liver function and carbohydrate mobilization have been investigated.

The blood sugar values during anesthesia and the period of recovery were studied for each of the above-mentioned drugs. The sugar values were determined by the Somogyi micro method (5) with the use of reagent no. 1 (6). The results are presented in tables 1, 2, 3, 4, 5 and 6.

The results from experiments with nembutal shown in table 1 do not include the $\frac{1}{2}$-hour series. With 110 observations for the control as well

[1] Nembutal (Pentobarbital)—Na Ethyl (1 methyl-butyl) barbiturate.
Pentothal—Na Ethyl (1 methyl-butyl) thiobarbiturate.
Seconal—Na Propyl-methyl-carbinyl Allyl Barbiturate.
Amytal—Na Iso amyl Ethyl Barbiturate.
Phenobarbital—Na Phenyl Ethyl Barbiturate.

as for the anesthesia series, there was no change in the mean blood sugar level (2). The mean deviation, however, was increased. In $\frac{1}{3}$ of the cases the blood sugar rose, in $\frac{1}{3}$ it went down and in $\frac{1}{3}$ there was no change. The most significant feature of these results is the fall in the sugar level at the time of recovery.

TABLE 1

*Blood sugar levels after nembutal; 40 mgm. per kilogram intraperitoneally. Normal-fed rabbits*

| | INITIAL | TIME AFTER INJECTION IN HOURS | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| Number of observations | 22 | 23 | 23 | 23 | 23 | 23 | 23 |
| Mean in mgm | 122 | 116 | 104 | 103 | 102 | 105 | 101 |
| Mean deviation $\epsilon$ | 22 | 15 | 11 | 14 | 12 | 11 | 14 |
| Mean deviation of mean $\epsilon_m$ | 5.0 | 3.1 | 2.3 | 2.9 | 2.5 | 2.3 | 2.9 |

TABLE 2

*Blood sugar levels after pentothal; 40 mgm. per kilogram intraperitoneally. Normal-fed rabbits*

| | INITIAL | TIME AFTER INJECTION IN HOURS | | | |
|---|---|---|---|---|---|
| | | $\frac{1}{2}$ | 1 | 2 | 3 |
| Number of observations | 41 | 49 | 47 | 48 | 46 |
| Mean in mgm | 111 | 125 | 115 | 111 | 100 |
| Mean deviation $\epsilon$ | 19 | 22 | 23 | 20 | 13 |
| Mean deviation of mean $\epsilon_m$ | 3.0 | 3.2 | 3.1 | 2.9 | 1.8 |

TABLE 3

*Blood sugar levels after seconal; 40 mgm. per kilogram intraperitoneally. Normal-fed rabbits*

| | INITIAL | TIME AFTER INJECTION IN HOURS | | | |
|---|---|---|---|---|---|
| | | $\frac{1}{2}$ | 1 | 2 | 3 |
| Number of observations | 37 | 33 | 33 | 32 | 32 |
| Mean in mgm | 99 | 107 | 104 | 95 | 94 |
| Mean deviation $\epsilon$ | 17 | 17 | 23 | 26 | 17 |
| Mean deviation of mean $\epsilon_m$ | 2.8 | 3.1 | 4.1 | 4.8 | 3.0 |

With pentothal, table 2, there was a rise in the sugar levels at the 20-minute and 1-hour intervals. Since the mean deviation changed but slightly, these rises may represent a real hyperglycemic effect of the pentothal. At the time of recovery, there was a significant fall in the blood sugar level.

Seconal, table 3, also seemed to raise the blood sugar levels at the $\frac{1}{2}$- and

1 hour intervals. Whether this represents a real hypoglycemic effect of the seconal or whether the small series of animals happened to include a majority of animals showing the hyperglycemia, is difficult to tell. The fall during the period of recovery is statistically significant but of less magnitude than that obtained with most of the other drugs. This drop is also illustrated in table 4.

With amytal, table 5, there was practically no change in the mean sugar values throughout the period of deep anesthesia; but the mean deviation was considerably elevated, indicating an increase in the variability. The fall at the time of recovery is significant since the mean deviation approaches the pre-injection value.

TABLE 4

*Blood sugar levels after seconal; 35 mgm. per kilogram intraperitoneally. Normal-fed rats*

| | INITIAL | AT THE TIME OF RECOVERY |
|---|---|---|
| Number of observations | 48 | 63 |
| Mean in mgm | 99 | 89 |
| Mean deviation $\epsilon$ | 7.5 | 10.5 |
| Mean deviation of mean $\epsilon_m$ | 1.1 | 1.3 |

TABLE 5

*Blood sugar levels after amytal; 40 mgm. per kilogram intraperitoneally. Normal-fed rabbits*

| | INITIAL | TIME AFTER INJECTION IN HOURS | | | | |
|---|---|---|---|---|---|---|
| | | ½ | 1 | 2 | 3 | 4 |
| Number of observations | 46 | 46 | 46 | 46 | 46 | 45 |
| Mean in mgm | 88 | 86 | 84 | 75 | 71 | 67 |
| Mean deviation $\epsilon$ | 13 | 20 | 20 | 16 | 11 | 11 |
| Mean deviation of mean $\epsilon_m$ | 1.9 | 6.7 | 3.1 | 2.3 | 1.6 | 1.7 |

With phenobarbital, table 6, there was no change in the first half hour but some fluctuation after 1 hour. Despite the fact that the animals were still anesthetized after the second hour, there was a significant fall in the blood sugar. Sugar values were not followed after 24 hours because of the superimposing effects of inanition.

Except in the cases of pentothal and seconal with which there were rises at the ⅓- and ½-hour intervals respectively, the mean blood sugar level after administration of these drugs remained practically unchanged at the time of deep anesthesia. Variability, however, was somewhat increased as evidenced by the larger mean deviations. Phenobarbital which produced anesthesia for at least 24 hours, caused no fall in the

sugar level during the first hour. In all cases there was, during recovery, a significant drop in the blood sugar levels. This is greater than can be attributed to inanition (7).

The fall in the blood sugar level during the period of recovery, together with the increased susceptibility to the drugs in fasting animals, led to a study of the relation of the various barbiturates to carbohydrate mobilization. As a measure of the glycogenolytic function of the liver, the rise in the blood sugar following the injection of epinephrine was employed. The normal blood sugar rises were taken from Coletti (8). In a previous investigation where the epinephrine response was tested at various times after the administration of nembutal, it was found that the greatest effect of the drug upon the epinephrine response occurred during the recovery period (4). Accordingly, 0.25 mgm. of epinephrine per kilo was injected subcutaneously at the beginning of recovery (after 24 hours in the case of phenobarbital) and the blood sugar values determined at the ½- and 1-hour intervals. The results are summarized in figure 1.

TABLE 6

*Blood sugar levels after phenobarbital; 150 mgm. per kilogram intraperitoneally. Normal-fed rabbits*

| | INITIAL | TIME AFTER INJECTION IN HOURS | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | ½ | 1 | 2 | 4 | 6 | 8 | 24 |
| Number of observations | 23 | 23 | 23 | 22 | 23 | 21 | 11 | 23 |
| Mean in mgm. | 94 | 95 | 98 | 87 | 86 | 80 | 83 | 75 |
| Mean deviation $\epsilon$ | 14 | 12 | 11 | 11 | 17 | 13 | 12 | 9 |
| Mean deviation of mean $\epsilon_m$ | 3.1 | 2.5 | 2.3 | 2.3 | 3.8 | 3.0 | 4.0 | 1.8 |

With each of the barbiturates studied there was a marked depression in the glycogenolytic power of the liver as shown by the absence or the diminution of the rise in the blood sugar after epinephrine. Short-acting barbiturates are detoxified in the liver and might be expected thereby to influence the glycogenolytic function of that organ. But phenobarbital which is eliminated by the kidneys likewise depresses liver function. This suggests that all barbiturates, regardless of their mode of elimination, temporarily impair liver function.

In order to determine whether the depression of glycogenolysis after epinephrine is specific for the barbiturates, or whether it may be the result of liver damage brought about by other means, liver degeneration was produced in 23 rabbits by the subcutaneous injection of chloroform. A solution of equal parts of chloroform and mineral oil was injected in the doses of 0.1 and 0.2 cc. per kilo for 3 successive days. Autopsies on representative animals killed the day preceding and on the day of the experiment revealed marked degeneration. Twenty-four hours after the third

injection, epinephrine was administered in the dosage of 0.25 mgm. per kilo, subcutaneously. Blood samples were taken just before the epinephrine administration, at the ½-hour and the 1-hour intervals after the injection. As would be expected, the initial blood sugar level was low, 76 mgm. From figure 1, it will be seen that the rise after epinephrine administration was very small.

Discussion. Whether or not it is permissible to use an anesthetic in the course of investigations on carbohydrate metabolism is a question constantly recurring to workers in this field. The results reported here indicate that barbiturates may be a disturbing factor in such studies. From the tables (1–6) it will be seen that although the mean sugar values

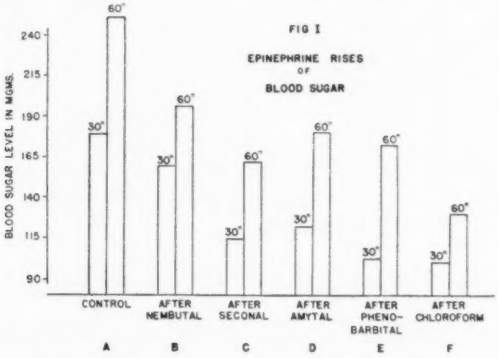


Fig. 1. Comparison of the blood sugar raising effects of epinephrine, 0.25 mgm. per kilo, in normal and in anesthetized animals.

A. Blood sugar levels at ½ and 1 hour after epinephrine in normal control animals.

Blood sugar levels at ½ and 1 hour after epinephrine in animals at the beginning of recovery from B—nembutal; C—seconal; D—amytal; E—phenobarbital.

F. Blood sugar levels at ½ and 1 hour after epinephrine in animals with liver damaged by chloroform.

do not change, the individual observations tend to fluctuate considerably at the time of anesthesia. The fluctuation is invariably reflected in an increased mean deviation. Very soon after the induction of the anesthesia there is a considerable fall in the blood sugar level which continues throughout the recovery period. This fall coincides with a progressive depression of carbohydrate mobilization. In an earlier paper (4), it was shown that even 30 minutes after the administration of nembutal the glycogenolytic power of the liver is already diminished. Amytal, which has been conceded to have little or no effect on carbohydrate metabolism, not only brings about an increased fluctuation of the blood sugar level at the time of anesthesia but also depresses the carbohydrate mobilization power of the liver. Phenobarbital, which is excreted by the kidneys and does not

involve the liver for its excretion, likewise depresses the glycogenolytic power of that organ. Diminished glycogenolysis appears to be the result of impaired liver function. The dysfunction induced by the barbiturates brings about the same impairment of carbohydrate mobilization as does that induced by chloroform.

SUMMARY

Some effects of various barbiturates upon carbohydrate metabolism in rabbits are presented. Nembutal and amytal produce no change in the mean blood sugar levels at the time of deep anesthesia; phenobarbital causes none in the first hour; pentothal and seconal bring about a rise in the first hour. All produce a fall in the blood sugar level at the time of recovery from anesthesia. All of the drugs studied cause a depression of the glycogenolytic power of the liver as evidenced by the relatively small effect of epinephrine on the blood sugar. The impaired glycogenolytic power of the liver is not related to the excretory processes taking place in the liver since phenobarbital, which is excreted by the kidneys, produces the same effect. This depression is probably not specific for the barbiturates, but may be the result of any liver damage since chloroform also causes the same depression.

The seconal used in this experiment was supplied by Eli Lilly and Co.

Grateful acknowledgment is made to Miss Ann Richardson and Mr. George Douglass for their assistance in the technical work.

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# THE RENAL TUBULAR REABSORPTION OF GLUCOSE IN THE NORMAL DOG

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Received for publication February 15, 1938

It is known that glucose is filtered through the glomerulus in the same concentration as it is present in the water of the plasma (Walker and Reisinger, 1933), and that its absence from the urine at normal plasma concentrations is due to the circumstance that it is reabsorbed by the tubule. In the amphibian kidney, this reabsorption is effected by the proximal segment (Walker and Hudson, 1937). In mammals the reabsorptive process is never complete, a small amount of glucose being present in the urine at normal plasma glucose levels (Harding, Nicholson and Archibald, 1936). Frank glycuresis at elevated plasma glucose levels is due, not to the complete cessation of reabsorption, but to the fact that more glucose is filtered than can be reabsorbed (Ni and Rehberg, 1930).

Ni and Rehberg (1930) have given a quantitative description of the reabsorption process, but we believe that their data are unsuitable for this purpose because venous blood was used for the calculation of the rate of glucose filtration in their experiments, because there were marked variations in the rate of filtration, and because significant errors were introduced by very rapidly changing plasma glucose concentrations. The present observations, also directed towards a quantitative description of the reabsorptive process, have been made in such a manner as to eliminate these sources of error.

EXPERIMENTAL PROCEDURE. Observations have been made upon two normal, well trained female dogs which were loosely restrained upon a comfortable animal board, and upon four dogs decerebrated under ether and chloroform anesthesia some hours prior to their use. Constant creatinine and varying glucose concentrations in the plasma were obtained by means of constant intravenous infusions. Urine collections were made by an inlying catheter, and at the end of each period the bladder was emptied as completely as possible and washed with warm water, this wash fluid being added to the urine prior to the final dilution for analysis. In the decerebrate dogs the ureter was cannulated and the urine delivered directly into a collecting vessel through a glass tube of small volume.

All bloods were obtained at the mid-period by puncture of the femoral artery.

CHEMICAL METHODS. Potassium oxalate was used in minimal quantities as an anticoagulant. The bloods were centrifuged immediately and the plasma precipitated within ten minutes of withdrawal from the animal, using the ferric sulphate-barium carbonate method (Steiner, Urban and West, 1932). The diluted urines were precipitated by the same method. Glucose was determined by the Folin (1929) method on plasma and urine filtrates diluted so that the actual concentration of glucose was close to 15 mgm. per cent. In the lower ranges of plasma glucose (i.e., below 500 mgm. per cent) all figures for urine glucose are given as the difference in copper reduction before and after absorption on yeast (Somogyi, 1928). Plasma and urine glucose has been taken as the observed glucose concentration minus 0.2 times the concentration of creatinine, a correction factor based on determinations of the reducing power of creatinine, using the above methods of precipitation and glucose analysis. The correction is of quantitative importance only in the range of plasma glucose at and below the level of frank glycuresis.

Creatinine determinations on plasma and urine filtrates were performed by the Folin and Wu (1919) method. The Jaffe reaction yields slight color with glucose in the concentrations involved in these experiments (Shannon, Jolliffe and Smith, 1932); we have avoided errors due to this circumstance by maintaining the plasma creatinine concentrations at 30 to 40 mgm. per cent, and reading the creatinine determinations in small groups between 10 and 14 minutes after the addition of the alkaline picrate. With these precautions the error in the determination of the creatinine clearance is negligible at moderate plasma glucose levels, and it does not increase appreciably at the higher glucose levels since the urine filtrates are diluted to the U/P ratio of creatinine, and consequently contain almost as much glucose as do the plasma filtrates. If the clearance of glucose or creatinine for any period, as calculated from each set of duplicate analyses, did not agree within two per cent, a third set was analyzed and the two showing best agreement were accepted. The values used for our calculations were the means of these duplicate analyses.

EXPERIMENTAL RESULTS. The experiments on normal dogs consisted of several groups of two or three observations made at successively higher plasma glucose concentrations. The procedure was such that the plasma glucose concentration was maintained at a nearly steady level in each group of observations and the creatinine clearance was essentially constant throughout the experiment. A typical series of observations of this type, made upon the normal dog, is given in table 1 and figure 1. A summary of five experiments is given in figure 2.

The absolute quantity of glucose reabsorbed by the tubules per unit

time is given by the difference between the rate of excretion and the rate of filtration of the sugar. This last term is given by the product of the plasma concentration and the creatinine clearance. Calculations made in this manner reveal that the tubular reabsorption of glucose is limited by the existence of a maximal rate. So long as the rate of filtration is such as to deliver glucose to the tubules at less than this maximal rate,

TABLE 1

*An experiment on a normal dog showing the relationship between glucose plasma concentration and its renal tubular reabsorption*

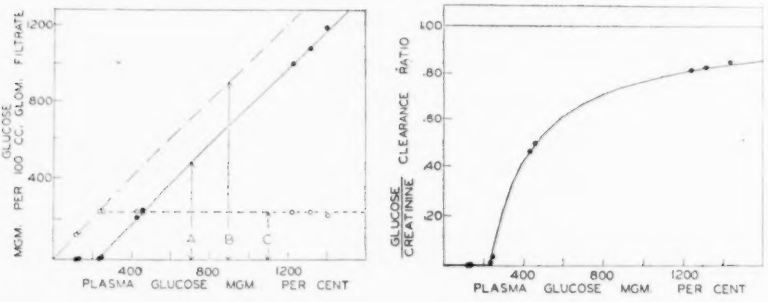
Dog C, 24.0 kgm.

| PERIOD | TOTAL CONCURRENT TIME | URINE FLOW | PLASMA LEVEL | | CLEARANCE | | CLEARANCE RATIO | GLUCOSE | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Creatinine | Glucose | Creatinine | Glucose | | Filtered | Excreted | Reabsorbed |
| | *min.* | *cc. per min.* | *mgm. per cent* | *mgm. per cent* | *cc. per min.* | *cc. per min.* | | *mgm. per min.* | *mgm. per min.* | *mgm. per min.* |
| | 0 | 1000 cc. water by stomach tube, 3 grams creatinine intravenously, infusion 5 per cent glucose, 0.55 per cent creatinine at 8 cc. per minute | | | | | | | | |
| 1 | 30–39 | 2.45 | 31.6 | 126 | 115.0 | | | 145 | 0.15 | 145 |
| 2 | 39–49 | 2.30 | 32.4 | 128 | 117.5 | | | 150 | 0.20 | 150 |
| | 50 | Infusion 10 per cent glucose, 0.55 per cent creatinine at 8 cc. per minute | | | | | | | | |
| 3 | 115–125 | 4.30 | 33.2 | 238 | 112.4 | 0.93 | 0.008 | 267 | 2.2 | 265 |
| 4 | 125–133 | 4.75 | 33.6 | 246 | 116.3 | 3.24 | 0.028 | 286 | 8.0 | 278 |
| | 134 | Infusion 20 per cent glucose, 0.55 per cent creatinine at 8 cc. per minute | | | | | | | | |
| 5 | 201–211 | 8.50 | 32.1 | 437 | 114.2 | 52.3 | 0.458 | 499 | 229 | 270 |
| 6 | 211–220 | 8.90 | 31.8 | 462 | 110.0 | 54.7 | 0.497 | 508 | 253 | 255 |
| | 221 | Infusion 20 per cent glucose, 0.20 per cent creatinine at 20 cc. per minute | | | | | | | | |
| 7 | 300–308 | 30.4 | 31.7 | 1234 | 121 | 98.2 | 0.812 | 1494 | 1212 | 282 |
| 8 | 308–317 | 29.0 | 31.5 | 1316 | 115.6 | 95.2 | 0.823 | 1521 | 1254 | 267 |
| 9 | 317–326 | 30.5 | 31.3 | 1410 | 117.0 | 98.8 | 0.844 | 1650 | 1393 | 257 |

reabsorption is essentially complete, as shown by the crossed symbols in figure 2, which indicate observations where less than one per cent of the filtered glucose is excreted. When delivery exceeds the maximal rate all the excess glucose is allowed to pass into the urine (see fig. 1a).

It is because of the existence of a maximal rate of reabsorption that the glucose clearance is essentially zero at low plasma levels, and that as the plasma level is raised glucose suddenly makes its appearance in

the urine, the glucose clearance thereafter rising and approaching the creatinine clearance (rate of glomerular filtration) as an upper limit (see fig. 1b). The point of transition in glucose reabsorption from the limitation imposed by the rate of filtration to the limitation imposed by the maximum rate of reabsorption is so abrupt that its exact experimental definition is very difficult or impossible. Individual experiments do show, however, that a small but significant amount of glucose first appears in the urine when the plasma concentration is 10 to 20 mgm. per cent below the level at which the maximal rate of reabsorption is reached.




*A* *B*

Fig. 1. Graphic analysis of the reabsorption and excretion of glucose (see table 1).

1a. Glucose filtration, reabsorption and excretion (mgm. per min.) per 100 cc. of glomerular filtrate. Curve *B*, the glucose filtered at various plasma concentrations; curve *A* (solid dots), rate of glucose excretion; curve *C* (open circles), rate of glucose reabsorption.

1b. In consequence of the relationship shown here, the glucose/creatinine clearance ratio (the fraction of filtered glucose which is excreted) rises as the plasma level is increased and approaches 1.0 as a limiting value. The curve is calculated on the assumption that 234 mgm. of glucose (per each 100 cc. of glomerular filtrate) can be reabsorbed.

The above experiments have consisted of successive observations made at progressively higher plasma glucose levels, the last observations being made at the highest plasma level. It is practically impossible to examine the reversibility of the reabsorptive system in such experiments by lowering the plasma glucose rapidly, nor can the examination be made on a falling plasma concentration following a single injection of a large quantity of glucose; the rapidity at which the plasma concentration falls, and the changes in glomerular filtration due to circulatory disturbance, so complicate the renal picture that such experiments are uninterpretable. It is possible, however, to obtain observations in a single experiment at high plasma glucose levels (800 to 1000 mgm. per cent) and then at low

levels (300 to 400 mgm. per cent); or at intermediate plasma levels (400 to 500 mgm. per cent) shortly after the elevation of the plasma glucose to this level (i.e., starting within 10 minutes) and again several hours later, the plasma concentration being maintained constant meanwhile by a constant intravenous infusion. Observations such as these invariably show a constant value for the rate of glucose reabsorption, and

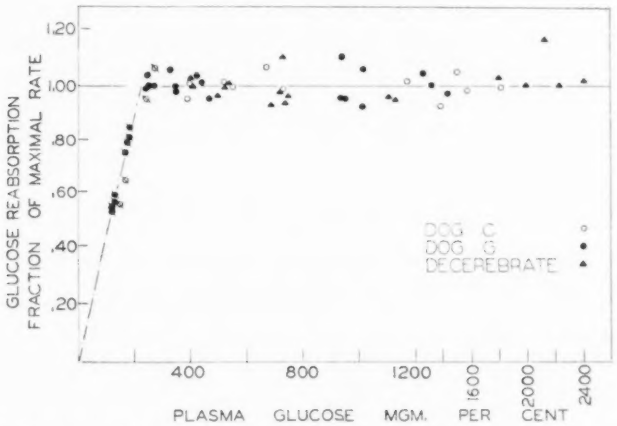



Fig. 2. Relationship between glucose reabsorption and plasma concentration and the genesis of the glucose threshold. The maximal rate of glucose reabsorption in each of seven experiments has been taken as the mean of the individual observations at plasma concentrations above the level of frank glycuresis. The rate of glucose reabsorption in each period of the experiment has then been expressed as the fraction of this mean value. The broken line shows the relationship to be expected in dog G, taking into account the mean rate of filtration and maximal reabsorption in this dog, and assuming that all filtered glucose is reabsorbed up to the maximal rate. The crossed symbols indicate observations where more than 99 per cent of the filtered glucose has been reabsorbed.

A curve calculated from equation 2, using K = 0.2, would follow closely the two lines in this figure. At plasma concentrations 20 mgm. per cent below the point of intersection of the two lines, reabsorption would be 99 per cent complete; at 20 mgm. per cent above the point of intersection the rate of reabsorption would be within 99 per cent of the maximal rate.

indicate that the reabsorptive mechanism is characterized by both physiological stability and reversibility. At no time have we discovered evidence that this mechanism is subject to "fatigue" in consequence of continued hyperglycemia.

Furthermore, the magnitude of the glucose reabsorptive maximum is fairly constant for any one dog over fairly long periods of time when the animal is maintained on the same diet, and it is not necessarily affected

by spontaneous changes in rate of glomerular filtration. Consequently glycuresis may occur at different plasma glucose levels in the same animal, not because of changes in the reabsorptive mechanism but because of changes in the rate of filtration and hence in the amount of glucose delivered to the reabsorptive system (Govaerts, 1936). A summary of data on two dogs examined over a period of five months is given in table 2.

Ni and Rehberg (1930) have suggested that the limiting factor in glucose reabsorption is a limit in the diffusion pressure which the tubule cells are capable of producing (or withstanding) between tubular urine and blood.[1] This question has been specifically examined in decerebrate dogs in which the kidney had been denervated and an adjustable clamp attached to the aorta just below the diaphragm.[2] The rate of glucose reabsorption

TABLE 2

*The maximal rate of glucose reabsorption in experiments separated by long periods of time*

Dog G: Weight 17 to 21 kgm. Surface area 0.78 sq.m. Kidney weight 148.5 g.
Dog C: Weight 26 to 24 kgm. Surface area 1.03 sq.m.

| DOG G | | | | DOG C | | | |
|---|---|---|---|---|---|---|---|
| Date | Number of periods | Filtration rate | Reabsorption maximum | Date | Number of periods | Filtration rate | Reabsorption maximum |
| | | *cc. per minute* | *mgm. per minute* | | | *cc. per minute* | *mgm. per minute* |
| 5–14 | 8 | 78.1 | 220 | 5–19 | 6 | 128.8 | 285 |
| 5–26 | 5 | 86.0 | 208 | 5–21 | 4 | 99.3 | 321 |
| 6–14 | 3 | 80.9 | 215 | 5–28 | 3 | 125.9 | 274 |
| 10–14 | 3 | 63.9 | 213 | 6–7 | 5 | 104.3 | 245 |
| 10–20 | 3 | 73.7 | 211 | 6–9 | 7 | 115.2 | 268 |
| | | | | 10–11 | 2 | 80.3 | 275 |
| | | | | 10–25 | 3 | 119.9 | 272 |

was determined at the normal filtration rate (using plasma glucose of 700 mgm. per cent), again after the filtration rate had been reduced by about 50 per cent by tightening the aortic clamp, and lastly after the

[1] In arriving at this conclusion Rehberg made the assumption that glucose reabsorption takes place prior to water reabsorption; if such were the case the existence of a maximal rate of reabsorption at a constant rate of glomerular filtration would produce a constant difference (diffusion pressure) between the tubular urine and the blood at various glucose plasma concentrations. This assumption is, however, unsupported by any evidence and possibly incorrect.

[2] The decerebrate dog yields the same results as the normal animal when subjected to an experimental routine similar to that in table 1. The results of two such experiments are shown as triangles in figure 2. These experiments indicate that decerebrate dogs may safely be used in the further examination of the glucose reabsorptive mechanism.

filtration had been restored to normal by removal of the clamp. If the limitation of glucose reabsorption were the diffusion gradient between the tubular urine and the blood, as suggested by Ni and Rehberg, the amount of glucose reabsorbed per unit time should have been lowered in proportion to the rate of filtration. As shown in table 3, the rate of glucose reabsorbed at normal and at reduced filtration rates was but slightly

TABLE 3

*An experiment on a decerebrate dog showing that the diffusion gradient established by glucose reabsorption cannot be the factor that limits this process*

Two hours before the start of the experiment ether anesthesia was induced and 20 minutes later operative procedure began, carotids tied, skull trephined, decerebrated; clamp applied to upper abdominal aorta; left ureter cannulated, right tied off; femoral arteries isolated, left connected to mercury manometer through a citrate system. Male dog; 14.0 kgm. Kidney weight 46.0 grams.

| PERIOD | TOTAL CONCURRENT TIME | URINE FLOW | PLASMA LEVEL | | CLEARANCE | | CLEARANCE RATIO | GLUCOSE | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Creatinine | Glucose | Creatinine | Glucose | | Filtered | Excreted | Reabsorbed |
| | *min.* | *cc. per min.* | *mgm. per cent* | *mgm. per cent* | *cc. per min.* | *cc. per min.* | | *mgm. per min.* | *mgm. per min.* | *mgm. per min.* |
| | 0 | 3 grams creatinine intravenously. Intravenous infusion 10 per cent glucose, 0.3 per cent creatinine at 4 cc. per minute. This was lowered slightly during second group of observations (4–6). Left femoral blood pressure 145–120 mm. Hg | | | | | | | | |
| 1 | 30–35 | 2.6 | 30.6 | 706 | 23.2 | 11.33 | 0.488 | 164 | 80 | 84 |
| 2 | 35–40 | 2.8 | 31.0 | 717 | 21.7 | 10.90 | 0.502 | 156 | 78 | 78 |
| 3 | 40–45 | 2.9 | 31.7 | 724 | 22.4 | 10.95 | 0.489 | 162 | 79 | 83 |
| | 50 | Aortic clamp tightened. Left femoral blood pressure 50–66 mm. Hg | | | | | | | | |
| 4 | 100–110 | 0.95 | 34.3 | 921 | 11.4 | 3.70 | 0.324 | 105 | 34 | 71 |
| 5 | 110–120 | 1.06 | 35.6 | 939 | 11.9 | 3.94 | 0.331 | 112 | 37 | 75 |
| 6 | 120–130 | 1.12 | 36.5 | 956 | 10.5 | 3.23 | 0.308 | 100 | 31 | 69 |
| | 135 | Aortic clamp released. Left femoral blood pressure 125–130 mm. Hg | | | | | | | | |
| 7 | 140–146 | 2.5 | 34.7 | 853 | 20.5 | 11.4 | 0.556 | 175 | 97 | 78 |
| 8 | 146–151 | 2.4 | 34.2 | 844 | 19.8 | 10.45 | 0.528 | 167 | 88 | 79 |
| 9 | 151–156 | 2.5 | 34.0 | 836 | 20.3 | 11.05 | 0.544 | 170 | 92 | 78 |

different, and we conclude that it is not the diffusion pressure, but an actual maximal rate, that limits the reabsorptive process.

Our observations on the dog kidney differ from those of Clark (1932) and Walker and Hudson (1933), who have concluded that the plasma glucose level is a specific determinant in the reabsorption of glucose by the amphibian kidney. The difference between the two animals may

be due to the method of examination, to differences in the permeability to the Amphibian and mammalian tubule, or to differences in the mechanism of glucose reabsorption. We are inclined to dismiss the last suggestion as improbable.

It should perhaps be stressed that our observations have all been made at relatively constant glucose plasma concentrations, under which conditions the reabsorptive system may be considered to be in kinetic equilibrium with the concentrations of glucose in the tubular urine and the renal interstitial fluid. This circumstance minimizes any error or physiological disturbance that might arise from rapid changes in the glucose concentration in either of these fluids.

Discussion. The demonstration that there exists a maximal rate in the tubular reabsorption of glucose is especially significant in view of the fact that the tubular excretion of many substances is limited in a similar manner. Certain aspects of tubular activity have been discussed elsewhere in terms of an hypothesis which treats the limitations of this activity in terms of the mass law (Shannon, 1938a). Applying this hypothesis to the present observations, we would suggest that in the process of reabsorption glucose enters into reversible combination with some element in the tubule cells, present in constant but limited amount, and that the subsequent decomposition of this complex limits the rate of glucose transfer from tubular urine to blood.[3] The conditions to be satisfied require two consecutive reactions:

$$(1) \qquad A + B \rightleftarrows AB \rightarrow T_r + B$$

where $A$ is the glucose in the tubular lumen, $B$ the cellular element, $AB$ the compound formed by the reversible combination of these two, and $T_r$ the glucose distal to the initial reaction. The equation which relates the arterial plasma concentration, $a$ (mgm. per cent), the rate of glucose reabsorption, $T_r$ (mgm. per min.) and the maximum rate of glucose reabsorption, $T_m$ (mgm. per min.) in terms of this hypothesis, is

$$(2) \qquad K = (a - T_r/V)\frac{(T_m - T_r)}{T_r}$$

where $K$ is the equilibrium constant and $V$ is the volume of glomerular filtrate in 100 cc. per minute. The application of this equation to our

[3] The data in the present report and that of other workers do not permit a further analysis of the reactions of transfer or the identification of the substance we have designated as $B$ in our equations. Nor do we feel it advisable to attempt such an analysis by analogy to the process of glucose transfer by the intestinal mucosa. The dissimilarity of the two processes and their inhibition precludes the use of such an analogy in the reconstruction of the reactions of transfer (see Lundsgaard, 1935; Verzar, 1936, and Walker and Hudson, 1937).

data is discussed in the legend of figure 2. The hypothesis appears to be as applicable to the tubular reabsorption of glucose as to the tubular excretion of various foreign substances. It is the limitation imposed upon the reabsorptive system by the amount of glucose contained in the glomerular filtrate, on the one hand, and the maximum rate of glucose reabsorption, on the other, that gives rise to the phenomenon of the "glucose threshold." According to (2) it is to be expected that small concentrations of glucose will appear in the urine at normal plasma glucose levels, that there will be little increase in the rate of excretion as the plasma level rises until the maximal rate of reabsorption is approached, and that the maximal rate of reabsorption will thereafter be rapidly attained (see legend, fig. 2). In view of the anatomy of the nephron it is recognized that as the tubular urine moves distally from the glomerulus there is progressive saturation of the reabsorptive capacities of the tubule cells and that frank glycuresis occurs only when the distal cells of the reabsorptive system are presented with more glucose than they are capable of reabsorbing per unit time. By taking the equilibrium concentration of glucose in the tubular urine to be $(a - T_r/V)$[4] we are neglecting the above fact; consequently $K$ in equation 2 has an artificially elevated value. This method of analysis is unavoidable at the present time, but adequate for the over-all description of the reabsorptive system.

SUMMARY

1. The tubular reabsorption of glucose has been examined by the simultaneous determination of glucose and creatinine clearances at various arterial plasma glucose concentrations in the normal dog.

2. The essential limitation in the reabsorptive process lies in the circumstance that the tubules are able to transfer only a certain maximal quantity of glucose from the tubular urine to the blood per unit time. When the rate of glomerular filtration of glucose is such that glucose is delivered to the tubules at less than this maximal rate, reabsorption is essentially complete.

3. This relationship has been discussed in relation to an hypothesis suggested to describe a similar limitation in the tubular excretion of certain substances.

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[4] Reabsorption of water in the proximal tubule would increase the concentration $(a - T_r/V)$ by some multiple which automatically becomes incorporated in the constant, $K$.

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# THE TUBULAR REABSORPTION OF XYLOSE IN THE NORMAL DOG

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Received for publication February 19, 1938

Our earlier belief that there is no active reabsorption of xylose by the renal tubules was disproved by the simultaneous investigations of Richards and his co-workers and ourselves on the excretion of inulin, which have shown that about 25 per cent of the filtered xylose is reabsorbed. Since this reabsorption is blocked by phlorizin it has been suggested that it is due to the participation of the pentose in the tubular mechanism for the reabsorption of glucose. The full evidence on the excretion of xylose and inulin in various vertebrates has been reviewed by Smith (1937) and need not be reiterated here.

One difficulty in the above interpretation is that, contrary to expectation by analogy with glucose, the fraction of filtered xylose which is reabsorbed is apparently independent of the plasma concentration. However, recent investigations on the reabsorption of glucose have served to clarify this point. It has been demonstrated that glucose reabsorption is limited in that the tubule cells can transfer only a certain maximal quantity of this sugar from urine to blood per unit time (Shannon and Fisher, 1938); this fact, with certain similarities in the tubular excretion of phenol red and other substances, has led us to a quantitative hypothesis of tubular activity (Shannon, 1938), and it is explicable under this hypothesis that a constant fraction of filtered xylose should be reabsorbed, as may be true in fact. Furthermore, it may be predicted from the hypothesis that elevation of the plasma glucose to levels slightly above the point at which frank glycuresis appears will completely, or nearly completely, block the reabsorption of the pentose. This paper is a report of observations on the simultaneous excretion of glucose and xylose designed to examine the tubular reabsorption of this sugar in this light.

The following considerations will serve to explain the purpose and design of the present experiments. In the reabsorption of glucose it is posited that the sugar combines reversibly with some cellular element

present in limited and constant amount, and that the reabsorptive process is limited by the rate of degradation of this complex;

(1)
$$\underset{\text{tubular glucose}}{A} + \underset{\text{cellular element}}{B} \rightleftarrows \underset{\text{cellular complex}}{AB} \rightarrow \underset{\text{distal glucose}}{T_r} + \underset{\text{liberated cellular element}}{B}$$

Assuming that the second of these reactions is a first order process, its rate being slow in relation to the attainment of equilibrium in the first, and stating them in terms of the law of mass action, we obtain the relationship:[1]

(2)
$$K = (a - T_r/V)\,\frac{(T_m - T_r)}{T_r}$$

where $a$ is the plasma arterial concentration (in mgm. per cent), $V$ the volume of glomerular filtrate (in units of 100 cc. per min.), $T_r$ and $T_m$ the submaximal and maximal rates of glucose reabsorption (in mgm. per min.), and $K$ is a constant.

It has already been suggested, on the basis of their chemical similarity and the action of phlorizin, that xylose and glucose are reabsorbed by a common mechanism. In terms of the above hypothesis and the derived equations, the rate of reabsorption of both sugars is limited by the availability of the common element, $B$.

A linear relation between $a$ and $T_r$ will exist in the reabsorption of any sugar (as for example, xylose) if the reaction $A+B \rightarrow AB$ is slower than the reaction $AB \rightarrow T_r+B$, since under these conditions the first reaction never attains equilibrium. In the case of xylose the overall velocity of such reactions must be slow as indicated by the low rate of xylose reabsorption. Or again, if the constant, $K$, is of large value, $T_r$ will appear to increase in proportion to $a$ over a wide range of the latter, since under this condition $T_r$ will approach the limiting value $T_m$ slowly. In either case, if both xylose and glucose combine with a common cellular element, $B$, and if the amount of $B$ present in the tubule cells constitutes the primary limitation in the rate of reabsorption of either sugar, which is the

[1] The derivation of this equation is given in full elsewhere (Shannon, 1938). In the case of glucose reabsorption below the plasma concentration of frank glycuresis, it is impossible to obtain a mean value to represent the equilibrium concentration along the tubule since the distal portion of the reabsorptive system is presented with very low concentrations of the sugar (Walker and Hudson, 1937). Consequently $(a - T_r/V)$ is taken as though the concentration at the end of the reabsorptive system were actually in equilibrium with the entire system. This results in an artificial elevation of the value of the equilibrium constant in equation 2. Reabsorption of water in the proximal tubule would increase the concentration $(a - T_r/V)$ by some factor which is incorporated in the constant K.

premise underlying equation 1, then the reabsorption of each of these sugars when in simultaneous competition for $B$ should be predictable from the separate examination of both systems. Such examination shows a great disproportion between the two constants; the order of magnitude of $K_{glucose}$ being 0.2 (Shannon and Fisher, 1938) and $K_{xylose}$ being 300 (see below). Consequently in the combined system at relatively low concentrations of glucose in the tubular urine $(a-T_r/V)$, nearly all the cellular element $B$ will be in combination with glucose, and negligible reabsorption of xylose will occur. The xylose reabsorbed should be reduced to practically zero at plasma glucose concentrations just above the level at which frank glycuresis occurs.[2] If xylose reabsorption occurs entirely by this active process, then the xylose clearance should under these conditions be equal to the rate of glomerular filtration. Again, the great disproportion between $K_{glucose}$ and $K_{xylose}$ should result in an essential linear depression of xylose reabsorption with increasing plasma glucose, if the plasma xylose is maintained at a low and constant concentration; and reducing the plasma glucose to hypoglycemic levels should not lead to any significant increase in xylose reabsorption since the quantity of $B$ available for this reabsorption will not be significantly increased by this procedure.

METHODS. Our experiments have been performed on normal female dogs. The experiments on the effect of plasma glucose on the reabsorption of xylose were performed upon the two dogs (C and G) that were previously used in the investigation on glucose reabsorption (Shannon and Fisher, 1938). On a mixed diet it was shown that the capacity of dog C to reabsorb glucose was 277 mgm. per minute and dog G was 213 mgm. per minute. In most instances constant xylose and creatinine plasma concentrations and varying glucose concentrations were obtained by continuous administration of appropriate infusions. In all experiments except those examining the reabsorption of xylose in relation to xylose plasma concentration, the plasma xylose concentration was maintained in the neighborhood of 100 mgm. per cent. In a few instances xylose was administered by stomach tube and creatinine by subcutaneous injection. Specific details of the experiments are given in the text and in table 1. The plasma glucose varied from 30 to 1000 mgm. per cent, but in any one group of observations it was either kept constant or allowed to change at a slow rate, since only under these conditions can the reabsorptive processes in the tubule be considered to be in kinetic equilibrium with the concentrations of xylose and glucose in the tubular urine.

[2] Some xylose may still be reabsorbed during hyperglycemia if the concentration of glucose in the distal portion of the tubule is insufficient to saturate the most distal reabsorptive cells, but this moiety would be difficult to discover since it would be small and within the experimental error.

The collection of blood and urine samples and the chemical determination of glucose and creatinine were carried out as described by Shannon and Fisher (1938). Xylose was determined as described by Jolliffe, Shannon and Smith (1932), but is expressed as xylose rather than as its glucose equivalent.

TABLE 1

*Showing the effect on xylose reabsorption of the saturation of the glucose reabsorptive mechanism by an elevated plasma glucose*

Experiment 36D, November 5, 1937. Dog G, weight 20 kgm., S.A.,0.81 sq.m. The clearance ratios starred are periods showing frank glycuresis.

| PERIOD | TIME | URINE FLOW | PLASMA CONCENTRATION | | | CLEARANCE | | CLEARANCE RATIO |
|---|---|---|---|---|---|---|---|---|
| | | | Glucose | Creatinine | Xylose | Creatinine | Xylose | $\frac{\text{Xylose}}{\text{Creatinine}}$ |
| | | *cc. per min.* | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* | *cc. per min.* | *cc. per min.* | |
| | 0 | 500 cc. water by stomach tube | | | | | | |
| | 10 | 3 grams creatinine and 5 grams xylose intravenously | | | | | | |
| | 15 | Infusion 6 cc. per minute. Creatinine 0.7 per cent; xylose 1.5 per cent | | | | | | |
| 1 | :31– :40 | 10.22 | 67 | 31.3 | 84.7 | 74.3 | 58.0 | 0.781 |
| 2 | – :51 | 9.63 | | 32.2 | 82.6 | 70.1 | 57.2 | 0.815 |
| 3 | –1:01 | 9.90 | 72 | 33.0 | 81.0 | 71.6 | 56.7 | 0.792 |
| | 1:02 | Infusion 6 cc. per minute. Creatinine 0.7 per cent, xylose 1.5 per cent, glucose 20 per cent | | | | | | |
| 4 | 1:30-1:40 | 11.20 | 403 | 34.0 | 74.3 | 76.8 | 79.8 | 1.040* |
| 5 | –1:49 | 10.85 | | 33.7 | 74.1 | 77.3 | 77.6 | 1.004* |
| 6 | –1:59 | 10.60 | 467 | 33.8 | 75.2 | 79.0 | 77.6 | 0.982* |
| | 2:02 | Infusion 6 cc. per minute. Creatinine 0.7 per cent, xylose 1.5 per cent, glucose 4 per cent | | | | | | |
| 7 | 2:25-2:36 | 5.82 | 304 | 34.7 | 82.6 | 69.3 | 64.9 | 0.937* |
| 8 | –2:45 | 5.30 | | 35.1 | 83.7 | 73.1 | 66.9 | 0.915 |
| 9 | –2:54 | 5.00 | 260 | 35.4 | 84.2 | 74.2 | 67.2 | 0.905 |
| | 2:57 | Infusion 6 cc. per minute. Creatinine 0.7 per cent, xylose 1.5 per cent, glucose 40 per cent | | | | | | |
| 10 | 3:19-3:28 | 14.00 | 807 | 33.2 | 78.3 | 79.3 | 81.8 | 1.032* |
| 11 | –3:48 | 14.20 | | 32.8 | 77.1 | 76.9 | 76.4 | 0.993* |
| 12 | –3:55 | 17.15 | 921 | 32.6 | 75.6 | 80.1 | 80.9 | 1.010* |

EXPERIMENTAL RESULTS. Preliminary experiments were performed to determine whether the use of constant intravenous infusions affected xylose reabsorption, as judged by the xylose/creatinine clearance ratio. It was found that the intravenous administration of 0.85 per cent saline to 20

to 24 kgm. dogs at the rate of 6.0 to 8.0 cc. per minute increased the rate of glomerular filtration, sometimes as much as 20 per cent; this increase in filtration rate was accompanied by an increase in the xylose/creatinine clearance ratio from the normal value of 0.72–0.76 to 0.79–0.83, but when the plasma xylose concentration remained constant the absolute rate of xylose reabsorption was not increased. The addition of glucose to the infusion fluid caused relatively little change in the filtration rate providing the glucose-free infusion was allowed to run long enough (usually from 30 to 50 min.) for the filtration rate to reach a constant value. The change in the xylose/creatinine clearance ratio, at constant xylose and varying glucose concentrations in the plasma can, therefore, be attributed to the effect of glucose on the reabsorption of xylose.

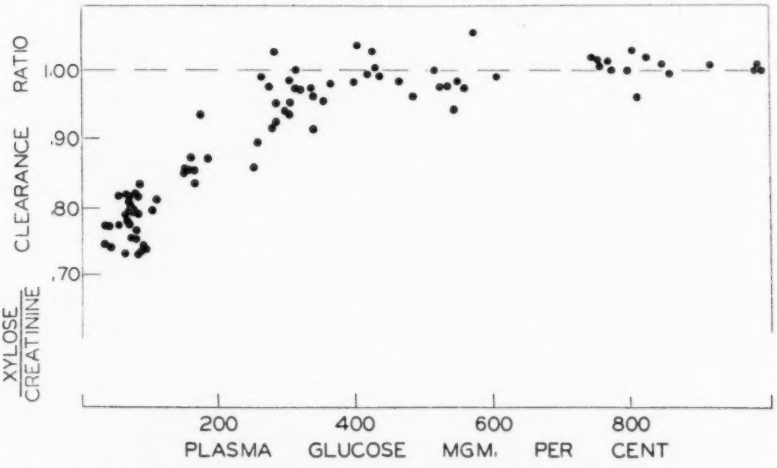


Fig. 1. Xylose reabsorption in relation to the plasma concentration of glucose. The fraction of filtered xylose which is reabsorbed is equal to 1.0-xylose/creatinine clearance ratio.

A second group of experiments was designed to examine the relation between the plasma concentration of xylose and the rate of tubular reabsorption. A typical experiment showed a rise in the xylose/creatinine clearance ratio from 0.80 to 0.90 when the plasma xylose was raised from 50 to 500 mgm. per cent, the creatinine clearance remaining constant. This change is so small that, considering the errors inherent in the determination of the ratio, no conclusions concerning the precise relation between plasma level of xylose and xylose reabsorption can be drawn. It seems doubtful if further observations of this nature can clarify this relationship, and we tentatively accept the belief that increasing the

plasma xylose concentration decreases slightly the fraction of filtered xylose which is reabsorbed; i.e., the absolute quantity reabsorbed increases with plasma concentration, but not in direct proportion to the latter. In terms of equation 2, $K_{xylose}$ would be of the order of magnitude of 300.

In a third group of experiments we examined the effect of elevating the plasma glucose upon xylose reabsorption. It is necessary to present these experiments in detail. Table 1 presents a typical experiment, and figure 1 a summary of all the experiments of this type. Xylose reabsorption is completely blocked (xylose/creatinine clearance ratio = 1.0) when the plasma glucose is raised to approximately 300 mgm. per cent, which is the plasma level at which frank glycuresis occurs in these dogs, and elevation of the plasma glucose above this level does not raise the xylose/creatinine ratio above 1.0. The xylose/creatinine clearance ratio appears to increase in a linear manner with increasing plasma glucose between levels of 60 and 300 mgm. per cent, but since the entire range of this ratio is from 0.75 to 1.00 it is impossible to define this relationship exactly. The xylose/creatinine clearance ratio at low glucose concentrations (30 to 40 mgm. per cent) obtained by small doses of insulin or during the spontaneous hypoglycemia following the intravenous administration of glucose, is not appreciably lower than at the normal glucose levels.

The above observations indicate that the hypothesis upon which equations 1 and 2 are based is applicable to the reabsorption of xylose in the normal kidney. It should be emphasized that equation 1 is not presented as a full description of the reabsorptive system, but is primarily directed toward a quantitative description of the observed limitation in this system. There is no reason to suppose that reabsorption of sugar by the renal tubules consists of a few simple, homogeneous and stoichiometric processes; in all probability the reabsorptive system is complex, heterogeneous and involves localized centers of activity. Equation 1 completely neglects the fact that the reabsorptive process involves the expenditure of energy, though it does imply that it is not a limitation in the energizing reactions which limit the rate of reabsorption in the normal animal. It is implicit in the first reaction, $A+B \rightleftarrows AB$, that this step in tubular reabsorption is effected at the expense of the free energy of the three reactants. However, the designation of the second reaction as a first order process does not preclude the possibility that this may be complex and involve an increase in free energy. The value of the hypothesis lies in the fact that it not only treats the reabsorptive process in a systematic and quantitative manner, but it reveals certain gross relationships which invite experimental examination, such as the prediction that elevation of the plasma glucose should completely block the tubular reabsorption of xylose. This observation is of special interest in that the identity of the xylose and

creatinine clearances in the normal dog during hyperglycemia supplies additional evidence that the inulin and creatinine clearances in the dog are at the level of glomerular filtration.

SUMMARY

1. The tubular reabsorption of xylose has been examined by the simultaneous determination of xylose and creatinine clearances at various arterial plasma glucose concentrations.

2. The elevation of the plasma glucose to the level of frank glycuresis completely and reversibly blocks the reabsorption of xylose.

3. It is concluded that in the dog all xylose is reabsorbed by an active tubular process which is identical with that responsible for the reabsorption of glucose.

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# UREA EXCRETION IN THE NORMAL DOG DURING FORCED DIURESIS

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Received for publication February 19, 1938

In a previous study of the excretion of urea in normal dogs it was shown that at the highest urine flow obtainable by water diuresis forty per cent of the urea filtered through the glomeruli was reabsorbed (Shannon, 1936). The present report is concerned with urea reabsorption at high urine flows obtained by forced diuresis.

The experiments were performed upon two normal dogs which had been used in the previous study. During the period of observation they were maintained on a mixed diet with an adequate salt and vitamine content. Constant creatinine and varying glucose, sulphate and urea concentrations in the plasma were obtained by constant intravenous infusions. All experiments were conducted in a manner similar to the one in table 1. In each experiment an effort was made to include observations over a wide range of urine flows. An observation was not considered valid when the rate of urine flow was changing rapidly, though frequently observations were accepted when the urine flow was slowly increasing. Most experiments were begun with the administration by stomach tube of 40 to 50 ml. of water per kgm., and the rate of the infusion was adjusted so that water and sodium chloride were replaced in amounts approximating their loss in the urine.

Blood and urine samples were collected as described by Shannon and Fisher (1938). In the glucose experiments femoral arterial blood was used for the analyses. Minimal quantities of potassium oxalate were used as an anticoagulant. The bloods were centrifuged and the plasma precipitated immediately upon withdrawal from the animal, using the ferric sulphate-barium carbonate method of Steiner, Urban and West (1932). The urines were diluted to the U/P ratio of creatinine and precipitated by the same method. Creatinine was determined by the Folin and Wu method (1919), urea by the Van Slyke urease method (1927) and glucose by the Folin method (1929). In the glucose experiments the precautions described by Shannon and Fisher (1938) were observed in the analysis of plasma and urine creatinine.

We were fortunate in having available for this investigation two of the dogs (C and G) which had been used in our previous work. Preliminary observations in the normal range of urine flows showed that the relationship between the urea/creatinine clearance ratio and the U/P ratio of creatinine had remained unchanged in the year that had elapsed since

TABLE 1

*An experiment showing the effect of extreme diuresis on the excretion of urea*

Experiment 22D, May 26, 1937. Dog G, weight 17.2 kgm., S.A. 0.78 sq. m., maintained on a mixed diet. The concentration of glucose in the urine can be obtained by multiplying the plasma concentration by the U/P ratio.

| PERIOD | TIME | URINE FLOW | PLASMA | | | U/P RATIO | | | CLEARANCE | | | CLEARANCE RATIO |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Creatinine | Urea | Glucose | Creatinine | Urea | Glucose | Creatinine | Urea | Glucose | Urea / Creatinine |
| | | | *mgm./100 cc.* | *mgm./100 cc.* | *mgm./100 cc.* | | | | *cc./min.* | *cc./min.* | *cc./min.* | |
| | 0 | 420 cc. water by stomach tube | | | | | | | | | | |
| | :30 | 420 cc. water by stomach tube, 250 mgm. per kilogram creatinine subcutaneously | | | | | | | | | | |
| 1 | 1:40–1:51 | 3.40 | 21.3 | 15.3 | 83 | 21.3 | 11.9 | | 72.5 | 40.5 | | 0.558 |
| 2 | –2:02 | 3.46 | 20.8 | 15.2 | 82 | 21.1 | 12.0 | | 73.0 | 41.5 | | 0.568 |
| | 2:05 | Infusion 6 cc. per minute 5.0 per cent glucose, 0.3 per cent creatinine | | | | | | | | | | |
| 3 | 2:45–2:58 | 3.62 | 31.8 | 14.4 | 173 | 21.3 | 13.2 | | 77.1 | 47.8 | | 0.621 |
| 4 | –3:08 | 3.10 | 31.4 | 14.0 | 151 | 24.5 | 15.46 | | 75.9 | 48.0 | | 0.632 |
| | 3:10 | Infusion 6 cc. per minute 10.0 per cent glucose, 0.4 per cent creatinine | | | | | | | | | | |
| 5 | 3:35–3:45 | 3.50 | 29.4 | 12.5 | 242 | 23.5 | 15.1 | .21 | 82.3 | 52.9 | 7.35 | 0.642 |
| 6 | –3:57 | 3.42 | 28.7 | 12.2 | 266 | 24.5 | 16.0 | .15 | 83.8 | 54.7 | 5.13 | 0.652 |
| | 4:00 | Infusion 6 cc. per minute 20.0 per cent glucose, 0.4 per cent creatinine | | | | | | | | | | |
| 7 | 4:25–4:36 | 12.35 | 28.7 | 10.8 | 520 | 6.79 | 5.21 | 3.52 | 83.9 | 64.4 | 43.5 | 0.767 |
| 8 | –4:45 | 13.55 | 28.2 | 10.7 | 551 | 6.51 | 4.90 | 3.79 | 88.3 | 66.4 | 51.4 | 0.752 |
| | 4:45 | Infusion 15 cc. per minute 20.0 per cent glucose, 0.16 per cent creatinine | | | | | | | | | | |
| 9 | 5:10–5:20 | 29.6 | 29.8 | 9.4 | 1178 | 3.01 | 2.64 | 2.40 | 89.6 | 78.7 | 71.5 | 0.877 |
| 10 | –5:32 | 30.0 | 30.7 | 9.3 | 1380 | 2.86 | 2.49 | 2.40 | 85.8 | 74.6 | 72.0 | 0.869 |
| 11 | –5:40 | 31.2 | 31.9 | 9.2 | 1500 | 2.66 | 2.37 | 2.19 | 83.0 | 74.0 | 68.4 | 0.892 |

previous examination. Our original data on these animals can, therefore, be used as a background for the presentation of our present results. In dog G we have obtained creatinine U/P ratios ranging from 700 during water deprivation to 1.75 during forced diuresis. This range in dog C is from 630 to 2.05.

A single experiment in which glucose was used as a diuretic, and which

follows essentially the same procedure as our experiments with sodium sulphate and urea, is given in table 1. The urea/creatinine clearance ratio (or the fraction of filtered urea which is excreted) in all experiments with forced diuresis is plotted against the log of the creatinine U/P ratio in figure 1, which includes our previous observations on these same animals.

The data divide themselves into two rectilinear or nearly rectilinear phases, one which extends from a creatinine U/P ratio of 2.0 to 10 or 20,

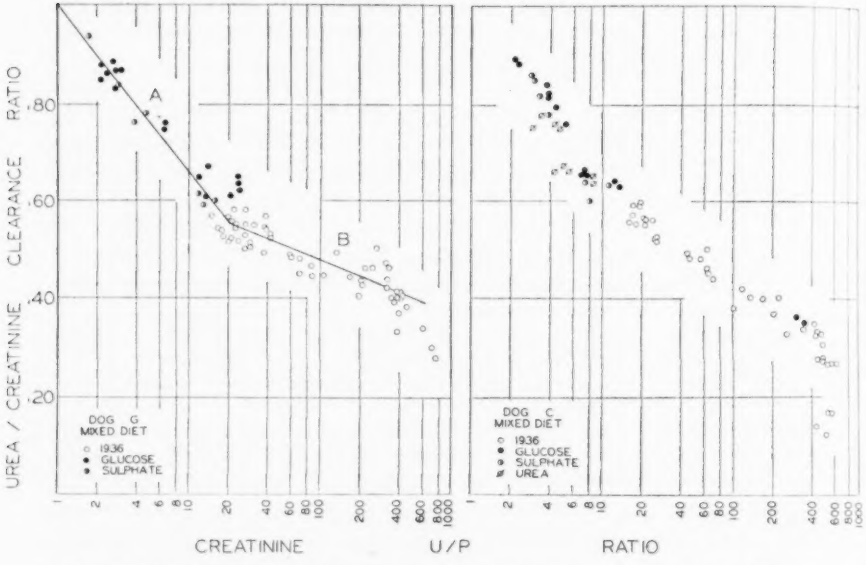


Fig. 1. The urea/creatinine clearance ratio in relation to the reabsorption of water as indicated by the U/P ratio of creatinine.

The fraction of filtered urea that is reabsorbed is equal to 1.0—the urea/creatinine clearance ratio. The open circles represent observations from our previous study on these dogs, maintained on a mixed diet, at normal urine flows and during water diuresis (Shannon, 1936).

and a second from a U/P ratio of 20 up to the highest values obtainable. Without implying any mathematical description of the data, these two phases have been emphasized in figure 1 by the two straight lines A and B.[1]

[1] It must be noted that rapid intravenous infusions, especially when the fluid is enriched with glucose or sulphate, may considerably increase the filtration rate, presumably because the plasma volume is expanded directly by the administered fluid and by drawing water from the tissues. This increase in filtration rate may be accompanied by a small increase in the urea/creatinine clearance ratio even where there is no significant change in urine flow, as shown in table 1. It is possible that

We have previously pointed out the inadequacy of a uniform process of diffusion, occurring distal to the site of water reabsorption, to explain the reabsorption of urea in the normal dog at creatinine U/P ratios above 20 (i.e., at urine flows of 0.05 to 5 cc. per minute). Our present observations lead us to extend this conclusion to creatinine U/P ratios of 2 to 20 (i.e., at urine flows of 5 to 50 cc. per minute). At a creatinine U/P ratio of 2.0, 20 per cent of the diffusion gradient, as calculated from bladder urine and blood, has already been dissipated, and yet when this ratio is increased to 20, only 46 per cent of the diffusion gradient is dissipated; if a uniform process of diffusion were involved, occurring distal to a single site of water reabsorption, the behavior of the system at a creatinine U/P ratio of 2.0 would lead us to expect that approximately 90 per cent of the diffusion gradient should be dissipated at the higher ratio. We are forced, therefore, to search for some mechanism to account for urea reabsorption which is in better agreement with the quantitative facts.

In considering the data in figure 1 there are four important points to be noted: *a*, the reabsorptive process is distinctly divisible into two phases, as indicated by lines A and B. *b*. The fact that line A extrapolates to 1.0 at a creatinine U/P ratio of 1.0 suggests that the reabsorptive process is not an active tubular process and may be due entirely to physical diffusion.[2] *c*. If increased physical diffusion is held responsible for the increased reabsorption occurring between creatinine U/P ratios of 20 to 700 (phase B), then the portion of the tubules in which this reabsorption occurs must be considered to be relatively impermeable to urea, since a relatively large increase in the time available for diffusion (which is proportional to the creatinine U/P ratio) results in only a small fractional reduction in the diffusion

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the elevation of the filtration rate from this cause has raised the urea/creatinine clearance ratios to some undetermined but slight extent in phase A. If so, the tendency for the data obtained with urea diuresis to fall below the other data may plausibly be explained by the fact that the urea infusions do not elevate the filtration rate, presumably because urea diffuses into the tissues.

We have not included in the above description the additional and variable lowering of the clearance ratio which is observed in severe dehydration, which may be incident to the lowering of glomerular filtration that accompanies this condition.

[2] These statements are not contradictory to those in the preceding paragraph. This moiety of urea loss, (A), is presumed to take place in the same segment as, and concurrently with water reabsorption, in consequence of the developing diffusion gradient of urea. The increase in time, in terms of the creatinine U/P ratio, available for diffusion under these conditions will be approximately half that calculated when water reabsorption occurs prior to any urea loss. The fractional dissipation of the diffusion gradient of urea in the range of creatinine U/P ratios of 2 to 10 is the expected order of magnitude if simple diffusion, under these conditions, is the mechanism of this urea loss. The data are such as to preclude closer analysis.

gradient. *d*. The portion of the tubule responsible for the reabsorption of urea at low U/P ratios (phase A) would appear to be more permeable to urea, since a small increase in the time available for diffusion leads to a relatively large fractional reduction in the diffusion gradient.[2]

In view of the above facts, we are led by inference to relate the reabsorption of urea to the reabsorption of water. Smith (1937) has recently suggested that the reabsorption of water in the mammalian nephron is divisible into two processes; first, an obligatory process which is essentially isotonic in nature, and which accompanies or is made possible by the active reabsorption of chloride and other osmotically active constituents from the tubular urine. This process presumably occurs in the proximal segment of the tubule. Second, the facultative or variable reabsorption of water which is potentially hypertonic in nature, and which is controlled by the antidiuretic hormone of the pituitary gland. This process presumably takes place in the distal segments of the tubule. No means is available at the present time for differentiating these processes in the mammal, but the hypothesis lends itself admirably to the explanation of our observations on urea reabsorption.

We may identify phase A in urea reabsorption with reabsorption in the proximal tubule; in this process a major portion of the water filtered is reabsorbed, and it is conceivable that in this process of concentrating glomerular filtrate, about 40 per cent of the filtered urea diffuses back into the blood. If the obligatory reabsorption of water (which is isotonic) is blocked by the introduction into the glomerular filtrate of osmotically significant quantities of glucose, sulphate or urea, the reabsorption of urea at this site fails to occur both because of a decrease in the extent to which urea is concentrated and because of a decrease in the time available for diffusion. We may identify phase B in urea reabsorption with the facultative reabsorption of water in the distal portions of the tubules, which may be presumed to be less permeable to urea. This is indicated by the smaller slope of line B as compared to line A. Since the obligatory reabsorption of water is completed in the normal animal at the highest urine flow obtainable by water diuresis, phase B in urea reabsorption invariably begins with a urine from which about 40 per cent of the filtered urea has already been reabsorbed. It is recognized that the two phases of urea reabsorption, as set forth above, are not subject to sharp separation or independent examination. It is probable that some urea reabsorption occurs in the distal portions of the tubule even when the creatinine U/P ratio is 10 or below. Since phase A extrapolates to a urea/creatinine clearance ratio of 1.0 at a creatinine U/P ratio of 1.0, physical diffusion may be tentatively accepted as the cause of reabsorption until evidence to the contrary is adduced.

The present observations on forced diuresis lend some support to Smith's

hypothesis. It is known that the dog's kidney can concentrate the urine above a $-\Delta$ 3.0°C., the normal range varying from 1.0 to 2.0°C. (unpublished observations). During glucose diuresis of the type reported here, the concentration of glucose in the urine is invariably below 5.0 per cent ($-\Delta$ of 0.517°C.) and may be lower at the higher urine flows than at the low urine flows. Since at these high urine flows the concentration of urea and chloride and other constituents is negligible, it is difficult to understand how the diuresis can be due to the osmotic pressure of the urine as it traverses the distal segments. But our results are explicable in the view that the presence of an unabsorbed, osmotically active constituent in the glomerular filtrate blocks the isotonic (obligatory) reabsorption of water in the proximal tubule, and that fluid is therefore delivered to the distal segments too rapidly for the reabsorptive system in this portion of the nephron to effect significant further concentration.

## SUMMARY

The excretion of urea has been studied in the normal dog by the simultaneous determination of creatinine and urea clearances during diuresis induced by the intravenous administration of glucose, sodium sulphate and urea. As the creatinine U/P ratio is reduced from 10 (the minimal value during water diuresis), progressively less urea is reabsorbed. The fact that the urea/creatinine clearance ratio extrapolates to 1.0 at a creatinine U/P ratio of 1.0 indicates that there is no active reabsorption of urea.

The reabsorption of urea is interpreted under the hypothesis that water reabsorption takes place at two sites in the nephron. The diffusion gradient created by the reabsorption of water in the proximal tubule accounts for the deficit in the urea clearance at the highest urine flow during water diuresis. The further deficit associated with low urine flows is attributed to diffusion in the distal portions of the nephron.

I wish to express my thanks for technical assistance to Dr. Elmer Alpert.

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# THE FLOW OF BLOOD IN THE CAROTID ARTERY OF THE DOG UNDER VARIOUS CIRCUMSTANCES AS DETERMINED WITH THE ELECTROMAGNETIC FLOWMETER[1]

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Received for publication December 13, 1937

Much less is known concerning the variations in blood flow than the pressure variations in the blood vessels. This has been remedied to a certain extent with the introduction of the Rein stromuhr (1) and the methods of Klisiecki (2) and Broemser (3).[2] Of these the Rein method has been the most satisfactory and has had the widest use. While these methods have been useful for the purposes for which they have been developed, there are limitations to their use for all purposes. Thus, the Rein stromuhr has several disadvantages which limit its usefulness in certain directions: 1, rapid fluctuations in flow cannot be recorded; 2, direction of flow cannot be determined; 3, the calibration of the flowmeter is cumbersome and the calibration curve is not a simple mathematical function; 4, the measurements and operation require specialized personnel; 5, the shape of the calibration curve in the region of slow flow introduces an ambiguity in the interpretation of the measured flow values; 6, the error of relative measurements is relatively high, being above 10 per cent, and 7, artefacts creep in readily (4).

Recently, a new type of flowmeter has been developed by Kolin (5 and 6) in this laboratory (and independently by Wetterer, 7) which does not possess the disadvantages of Rein's method. It is based on the principle that in blood moving through a magnetic field at right angles to the magnetic lines of force an induced e.m.f. will be established perpendicular to the magnetic field and at right angles to the direction of blood flow. This induced voltage is proportional to the velocity of blood flow and can be tapped by placing non-polarizable electrodes on the unopened vessel wall. The amplified voltage can be recorded by means of an oscillograph which will furnish an undistorted record of the cyclic variations in flow provided

[1] Aided by a grant from Mr. L. Sigman and the A. B. Kuppenheimer Fund.

[2] A hot wire method has been described recently by T. E. Machella (This Journal 1936, **115**, 632).

an interrupter is placed in the input (a 50 cycle tuning fork was used in these experiments). By planimetry of the records the mean rate of flow in cc./sec. can be determined. The calibration can readily be carried out by injecting saline solution or freshly drawn blood into the blood vessel on the peripheral side of the flowmeter, the vessel being clamped peripherally from the needle. A simultaneous record (viz. fig. 2 E) permits the evaluation of this injection in cc./sec. (and cm./sec.), provided the blood vessel is encased in a sleeve to prevent change of its cross section at the point where the electrodes are applied. One injection determines one point of the calibration curve which is a straight line going through zero so that the calibration is thereby completed. The details of the method are described in the appendix.

In the present report, we wish to record our experience with this flowmeter in registering the flow in the carotid artery under various circumstances, viz., *a*, normal conditions under anesthesia with chest closed and chest open; *b*, during intravenous saline infusion; *c*, during partial occlusion of the thoracic aorta and following release; *d*, during experimental production of incompetence of the aortic valve; *e*, during changing heart rate, and *f*, during ectopic beats and gasping respiration.

A. *Velocity pulse in the carotid artery under normal conditions in the anesthetized animal with chest closed and chest open.* At the rate at which the dog heart was beating, the velocity pulse in all the experiments consisted of two positive waves, a primary one (systolic) and a secondary one (diastolic) (cf. figs. 1 A, 2 A and B, 5). The dimensions and contour of these two waves are clearly depicted, and some of the quantitative data are shown in the legends and in table 1. The flow per beat can be subdivided into the flow during the inscription of the primary wave and that during the secondary wave, and these constitute variable proportions of the flow per beat within the range depicted. Four points on the velocity pulse are easily determined, viz., the onset and peak of the primary wave and the onset and peak of the secondary wave. It will be seen that the peak of the primary wave is always considerably higher than the peak of the secondary wave, and that at the rapid heart rates encountered, the onset of the primary wave is higher than the onset of the secondary wave. We have not seen a curve, at the start of any of these experiments, in which a back flow in the carotid artery could be demonstrated during the heart cycle.

The velocity curve recorded in this manner gives a time picture of the velocity of flow from moment to moment during the heart cycle which by integration can give the total volume flow during the heart cycle or per minute, as well as during the various phases of the heart cycle. This curve also gives the fluctuations in the pressure gradient existing in the artery at the point where the electrodes are placed. Our results show that

this time record of the pressure gradient at a point has a different contour than that given by the pressure-time curve ordinarily recorded by pressure manometers. The velocity pulse differs, as we can see, from the pressure pulse in two important respects: 1, it does not show the fine vibrations seen in the latter, and 2, the onset of the primary wave of the velocity pulse contrary to the pressure pulse is at a higher level than the onset of the secondary wave. It is apparent that the velocity pulse when recorded simultaneously with the pressure pulse can give the data from which can be computed the energy content of the blood passing the flowmeter in terms of potential and kinetic energy (Katz, 8). The pressure pulses recorded simultaneously with the velocity pulse will also furnish the exact time relations of the latter to the phases of the heart cycle.

TABLE 1

*Blood flow in the carotid artery*

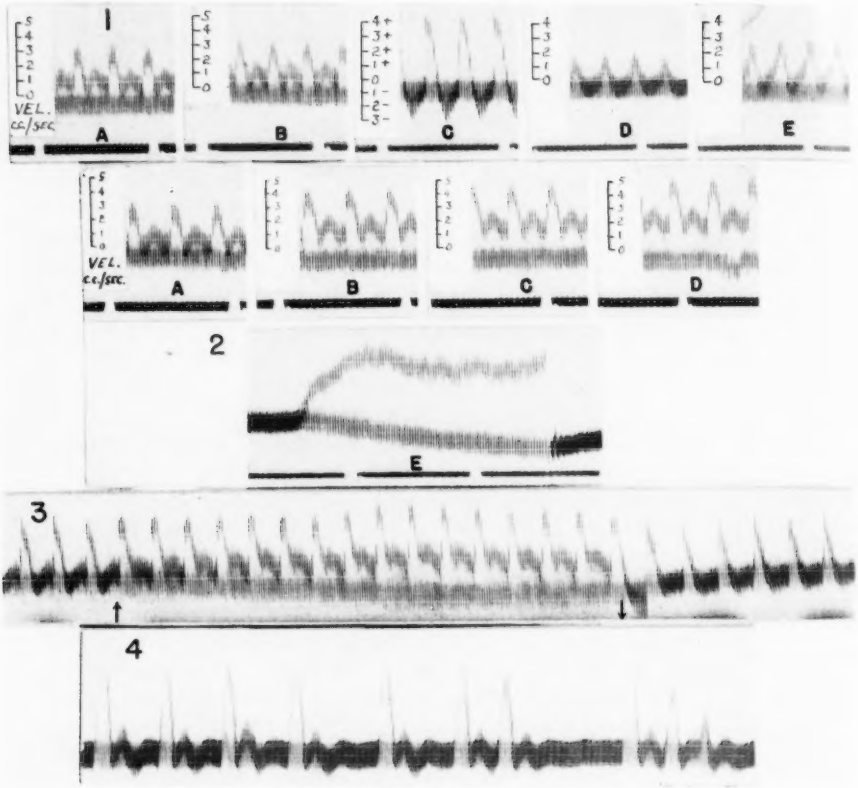
| EXPERIMENT NUMBER | CONDITION OF ANIMAL | HEART RATE | MEAN FLOW | TOTAL FLOW | TOTAL FLOW DURING PRIMARY WAVE | TOTAL FLOW DURING SECONDARY WAVE | VELOCITY AT VARIOUS POINTS OF CURVE | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Onset of primary wave | Peak of primary wave | Onset of secondary wave | Peak of secondary wave |
| | | *beats per min.* | *cc. per min.* | *cc. per beat* | *cc. per beat* | *cc. per beat* | *cc. per sec.* | *cc. per sec.* | *cc. per sec.* | *cc. per sec.* |
| 1 | Chest closed | 132 | 203 | 1.54 | 0.91 | 0.63 | 3.3 | 9.2 | 1.9 | 4.8 |
| 2 | Chest closed (fig. 1A) | 222 | 124 | 0.56 | 0.31 | 0.25 | 1.5 | 4.8 | 1.2 | 2.2 |
| 2 | Chest open, artificial respiration (fig. 1B) | 207 | 77 | 0.37 | 0.21 | 0.16 | 1.0 | 2.8 | 0.7 | 1.1 |

A comparison of the velocity pulses at various rates of flow shows that the amplitude of the primary wave at slower flows is reduced more than that of the secondary wave. Such changes occur in an animal when the chest is opened, apparently because of the associated hemorrhage and trauma (cf. table 1 and fig. 1 A and B). This is due to the decrease in venous return decreasing the cardiac output.

B. *Velocity pulse in the carotid artery during saline infusion.* Intravenous infusion of saline (at body temperature) at a fairly rapid rate raises the level of the onset of the waves and increases the height of both the primary and secondary waves of the velocity pulse, the former more than the latter (cf. fig. 2). The changes in circulating blood volume and cardiac output accompanying saline infusion (and lasting for some time after the infusion is ended) and the hemorrhage and trauma of opening

the chest thus are seen to affect the flow during systole more than that during diastole.

C. *Velocity pulse in the carotid artery during partial occlusion of the thoracic aorta and following release.* A typical experiment is shown in figure 3. The velocity pulse before the thoracic aorta was partially occluded is characteristic of the type encountered when the heart rate was rapid and the arteries poorly filled. The peak of the primary wave occurs early after its onset, and its summit then descends more slowly. The onset of the primary wave occurs before the completion of the secondary wave which gives the diastolic portion of the curve an ascending inclination. When the thoracic aorta is partially occluded (indicated by upright arrow), the velocity pulse changes in several characteristics: 1, the base of the pulse rises progressively; 2, the top of the primary wave becomes an ascent and the peak occurs later; 3, the secondary wave now starts at a higher instead of a lower level than the primary wave so that the diastolic portion of the pulse becomes a descent instead of an ascent; 4, the duration of the secondary wave shortens so that it is completed before the primary wave begins. Unlike the effect of saline infusion, the amplitude of the primary wave is not greatly increased, (its onset rises as much as its top), so that a greater porportion of the flow increase occurs during diastole. After release of compression (inverted arrow), the velocity pulse tends to resume its former contour. However, at the moment of release, there is a clear indication of a temporary back flow in the carotid artery lasting 0.18 sec. and indicated by the flow curve going below the zero level. Obviously, this last is due to the reversal of the pressure gradient at the moment when the aorta is released. The pressure in the carotid artery on release of the occlusion of the aorta is at first considerably higher than in the aorta below the occlusion causing the temporary reversal in the pressure gradient from aorta to carotid artery. The change in contour of the carotid velocity pulse is to be expected from the change in its pressure pulse which has been reported to follow aortic occlusion (Katz and Siegel, 9; Wiggers, 10). The increase in the flow in the carotid artery is attributed to the diminution of flow in the aorta caused by the occlusion. The change in the contour of the primary wave is evidence of the change in the manner in which the left ventricle ejects its blood, and this is shown also in the change of contour of the intraventricular pressure curve (Katz and Wiggers, 11). The diastolic rate of flow is increased more than the systolic as shown by the fact that when the resistance is increased, the increase in amplitude of the primary wave is less than the increase of the average height of the secondary wave above zero. This is due to the elastic reservoir function of the large systemic arteries, permitting them to store more potential energy during




Figs. 1–4

Fig. 1. Velocity pulses in the carotid artery of an anesthetized dog during the control period with chest closed (*A*), and with chest open (*B*), and following the production of an insufficiency of the aortic valve of relatively large (*C*) and small degrees (*D* and *E*). Calibrations at the left of each segment give the velocity in cc./sec. Time is in seconds. In this and succeeding curves, the record shows the 50 cycle vibrations of the tuning fork used to interrupt the input. The top of the curve should be used and related to the top of the base line which is zero flow. Discussed in text.

| SEGMENT | HEART RATE | FLOW PER HEART BEAT | TOTAL FLOW DURING PRIMARY WAVE | TOTAL FLOW DURING SECONDARY WAVE |
|---|---|---|---|---|
| | *beats per min.* | *cc.* | *cc.* | *cc.* |
| A | 222 | 0.56 | 0.31 | 0.25 |
| B | 207 | 0.37 | 0.21 | 0.16 |
| C | 207 | 0.27 | 0.31 | −0.04 |
| D | 240 | 0.28 | | |
| E | 244 | 0.33 | | |

systole, when the resistance is increased, to be available for conversion into kinetic energy of diastolic flow. The change in the duration of the secondary wave following occlusion of the aorta could be explained by an increase in the natural period of the carotid artery, when the pressure within the artery is elevated, and the associated change in the velocity of propagation of the reflected waves. In the determination of the final form of the velocity pulse, the change in the phase relationship between the centrifugal and reflected waves plays an important rôle. This type of experiment suggests that the contour of the velocity pulse depends in part

Fig. 2. Velocity pulse in the carotid artery of an anesthetized dog during rapid saline infusion. Segment *A* is the control (chest closed). Segments *B*, *C* and *D*, various stages of saline infusion. (In segment *D* the sudden shift in the base line is an artefact.) Segment *E* is a calibration curve during which 10 cc. of saline were injected with an 18 gauge needle directly into the carotid artery cephalad to the flowmeter, the carotid artery being clamped off cephalad to the needle which eliminated the cardiac pulsations.[3] This was repeated 3 times; the planimeter areas obtained were 9.1 sq. cm. (in the curve illustrated), 8.2 and 8.5 sq. cm., giving an average of 8.6 sq. cm. calibrations in cc./sec. Time in seconds. Discussed in text.

| SEGMENT | HEART RATE | TOTAL FLOW PER BEAT |
|---|---|---|
| | *beats per min.* | *cc.* |
| A | 187 | 0.48 |
| B | 177 | 0.78 |
| C | 194 | 0.81 |
| D | 187 | 0.85 |

Fig. 3. Velocity pulse in the carotid artery of an anesthetized dog with chest open showing the effect of almost complete occlusion of the thoracic aorta (upright arrow) and release (inverted arrow). The time of each double vibration is 1/50 sec. Discussed in text (calibrations in table below in arbitrary units).

| | TOTAL FLOW PER BEAT | TOTAL FLOW PER PRIMARY WAVE | TOTAL FLOW PER SECONDARY WAVE | VELOCITY AT | | VELOCITY AT | |
|---|---|---|---|---|---|---|---|
| | | | | Onset | Peak | Onset | Peak |
| | | | | Of primary wave | | Of secondary wave | |
| | *cm.*³ | *cm.*³ | *cm.*³ | *mm.* | *mm.* | *mm.* | *mm.* |
| Second beat of record | 0.27 | 0.18 | 0.09 | 0.5 | 13.0 | 0 | 4.5 |
| Tenth beat after upright arrow | 0.80 | 0.44 | 0.36 | 6.5 | 19.5 | 9 | 10.5 |

Fig. 4. Velocity pulse in carotid artery of an anesthetized dog with chest open during marked arrhythmia (presumably sinus in origin) at the height of epinephrine action. The time of each double vibration is 1/50 sec. Discussed in text.

[3] This indicates that the pulsations are due to flow and not to pressure or volume changes in the artery.

on the length, tension, diameter and elastic properties of the vessel wall on both sides of the flowmeter.

D. *The velocity pulse in the carotid artery in experimental production of incompetence of the aortic valve.* A typical experiment is shown in figure 1 (segments B, C, D and E are from a continuous strip). The aortic incompetence was produced in the manner described by Wiggers (12). The perforated cannula used by us was 6 mm. in internal diameter. It will be seen that the production of aortic incompetence produced several characteristic changes (compare segments B and C of fig. 1, also segment B of fig. 8 with segments A and C): 1, it lowered the level from which the primary wave started; 2, it increased the amplitude of the primary wave and increased the celerity of the rise and fall of this wave; 3, it increased the total flow during the time this wave was inscribed (the heart rate being the same); 4, the secondary positive wave was replaced by a negative wave having its trough soon after the end of the primary wave, thus producing a definite back flow during diastole amounting in this case to 0.04 cc. per beat or 13 per cent of the net flow during the beat (in other instances the back flow was even greater, reaching as much as 30 per cent). As suggested by Wiggers (12), we found that the greatest rate of back flow occurs early in diastole and then fades off. When the perforated cannula in this particular experiment was retracted so that none of it protruded past the aortic valve, the contour of the curves suggested that the aortic leak remained perhaps because of tears in the aortic valve (as verified post mortem). Yet in segments D and E of figure 1 the leak was so tiny that no back flow could be demonstrated. In other experiments, the curve after removing the leak returned to its previous contour (cf. fig. 8). This flowmeter method has for the first time actually demonstrated by graphic record the back leak which has been described in incompetence of the aortic valve. Our experiments demonstrate that aortic incompetence will produce a back flow in the carotid artery only when the incompetence is of sufficient magnitude, otherwise the incompetence merely reduces the flow during diastole.

E. *The velocity pulse in the carotid artery during marked arrhythmia of the heart.* On several occasions marked cardiac arrhythmia, presumably sinus in character, was noted while recording the velocity pulse of the carotid artery. Usually this occurred during peripheral vagus stimulation and occasionally it followed epinephrine injection. Figure 4 represents an experiment of the latter sort. In this experiment the arteries were fairly empty as shown by the small magnitude of the diastolic flow. It will be seen that the amplitude of the primary wave varied with the cycle length (measured from the beginning of the preceding diastole to the beginning of the following diastole), and when this is correlated in a graph (fig. 6), a non-linear curve is obtained, resembling that correlating duration

of mechanical systole to cycle length (Katz, 13). In contrast with this, the total flow during the inscription of the primary wave, in this instance constituting most of the cycle flow, varies in a linear fashion with the cycle length in the range studied (fig. 6). Since in this instance the flow during the primary wave constituted almost the entire flow per beat, the flow per beat is very nearly proportional to cycle length. It follows that the flow per beat divided by the cycle length (which is flow per unit time) remains constant, regardless of the irregularity of the heart. This means that over this period of time the mean flow has remained practically constant.

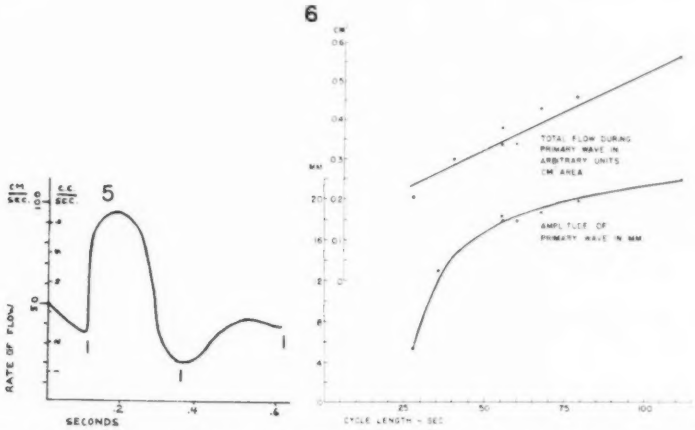
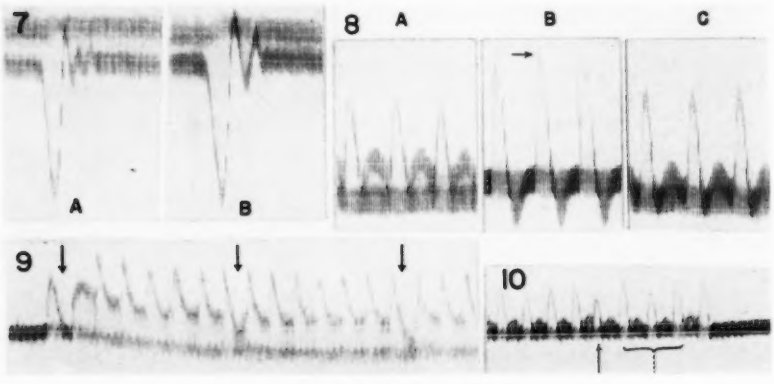



Fig. 5. Velocity pulse of carotid artery retraced to give its dimensions. This curve was obtained from an anesthetized dog after the chest was opened. Discussed in text.

Fig. 6. Relation of amplitude and area of primary wave of the velocity pulse to the duration of the preceding cycle length. Discussed in text.

Another interesting observation depicted in figure 4 is the undulations of the diastolic portion of the curve. It will be seen that following the secondary wave the curve goes below the base line showing evidence of a back flow. The waves which succeed this secondary wave show two characteristics which are seen best in the long pause toward the end of the figure, viz., 1, a progressive decay in amplitude, and 2, a progressive increase in the duration of the successive waves. This type of curve could be expected if the primary wave set up a series of free oscillations in the carotid artery; the change in wave duration would be anticipated as the artery became emptied of blood since the natural period of the artery becomes longer as the artery becomes less distended, and this reduces the rate of propagation of the reflected waves. A similar series

of secondary waves was obtained following cessation of flow in an artificial model (see fig. 7 B), except that here the period of the waves remained approximately constant since the vessel was not permitted to empty. When a sleeve was placed around the isolated artery, the curve became somewhat more complex (fig. 7 A).




Figs. 7–10

Fig. 7. Velocity pulse obtained from an isolated length of the dog's carotid artery, obtained postmortem and made part of an artificial circulation scheme, to show the oscillations which follow cessation of flow when a sleeve is placed around the artery (*A*) or is omitted (*B*). Discussed in text.

Fig. 8. Velocity pulse in the carotid artery of an anesthetized dog with chest open showing the effect of insufficiency of the aortic valve. *A* and *C* are controls before and after production of the aortic incompetence, *B* is the pulse during the aortic incompetence. Discussed in text.

Fig. 9. Velocity pulse in the carotid artery of an anesthetized dog with chest closed showing the effect of gasping respiration on the carotid blood flow. Gasps are indicated by arrows. Discussed in text.

Fig. 10. Velocity pulse in the carotid artery of an anesthetized dog with chest open showing effect of premature systole (indicated by arrow), in which an alternans of the velocity pulse followed the extrasystole (indicated by bracket). Discussed in text.

These observations suggest that the velocity pulse is modified by the same factors that affect the pressure pulse (Wiggers, 14).

F. *Velocity pulse in the carotid artery in ectopic beats and during gasping respiration.* In figure 9 is shown a velocity pulse taken on an animal whose natural respiration was gasping in character. The gasps are indicated by the arrows. Every gasp is accompanied by a sharp dip in the velocity pulse about half the amplitude of the primary cardiac wave. Apparently the pressure within the thoracic aorta is reduced markedly by the drop

of the intrathoracic pressure during the gasp so that there is a considerable diversion of the blood from the carotid artery to the aorta. This is, however, not great enough to produce a back flow. This experience supports the emphasis laid on the importance of abnormal respirations on the dynamics of the arterial system which was suggested by Hamilton et al. (15). These changes operate also during normal and artificial respiration, since cyclic variations in the amplitude of the primary wave were noted a number of times.

Premature beats were found to cause a decrease in the primary wave of the velocity pulse roughly in proportion to their prematurity. However, we have not had a sufficient number of them as yet in any single record to establish accurately the relationship to prematurity such as was demonstrated for sinus arrhythmia in figure 6. On several occasions, however, we found that a single premature beat established a transitory alternans of the succeeding velocity pulses. An example is illustrated in figure 10.

## APPENDIX

### *Electromagnetic rheometry and its application to blood flow measurements*

A. KOLIN

*Principles.* The operation of the electromagnetic flowmeter depends on the fact that when an electrical conductor moves through a magnetic field at right angles to the lines of magnetic force, a potential difference is created within the conductor at right angles to both the direction of the magnetic lines of force and the direction of motion of the conductor. Thus if the ends of the conductor are connected through an external circuit, a current will flow. The magnitude of the induced electromotive force depends on the strength of the magnetic field, the extent of the conductor in the direction of the potential gradient and the velocity of motion of the conductor in the direction at right angles to the lines of magnetic force; it is independent of the nature of the material composing the conductor. Mathematically it may be expressed:

$$E = H \cdot l \cdot v \cdot 10^{-8} \qquad (1)$$

where $E$ is the electromotive force set up in volts, $H$ is the magnetic field strength in oersted, $l$ is the length of the conductor in centimeters at right angles to the direction of motion and the direction of the magnetic lines of force, and $v$ is the velocity of motion of the conductor in cm./sec. in a direction at right angles to the lines of magnetic force.

As soon as the conductor moves out of the magnetic field, the electromotive force of course vanishes. If, however, the conductor be an electrolyte streaming in a tube of constant diameter between the magnet poles, a potential gradient will be maintained in the electrolyte as long as

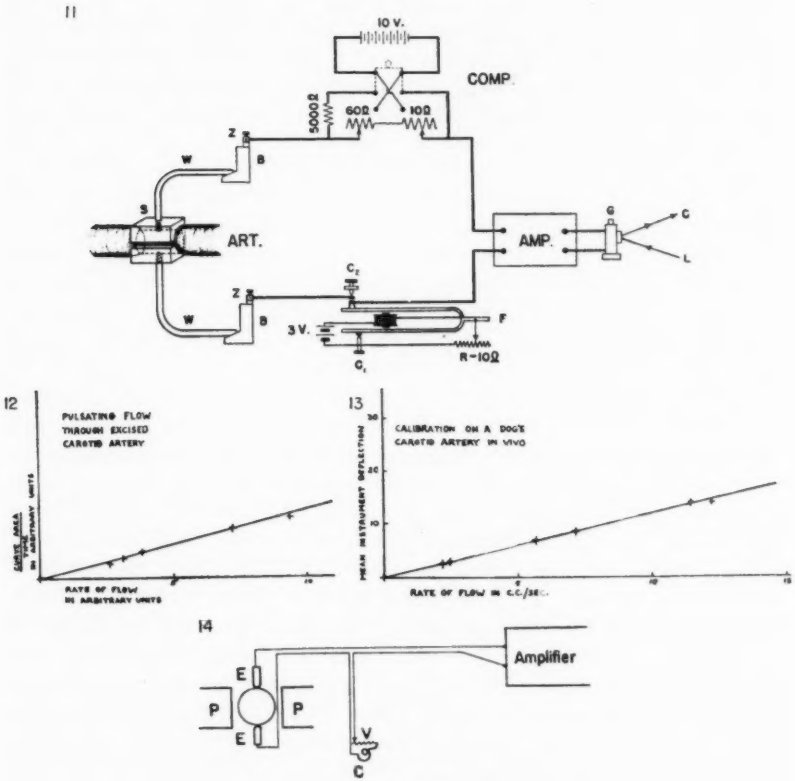
it is flowing. In this case equation (1) above also applies, but if the strength of the magnetic field and the diameter of the flowing stream at the point where the recording electrodes are attached are both maintained constant, the resulting electromotive force will vary only with the velocity and in a linear fashion:

$$E = (H \cdot l \cdot 10^{-8})v = Kv \qquad (2)$$

where $l$ is the internal diameter of the tube and $E$ is the potential difference at the ends of a diameter which forms a right angle with the lines of force. Thus, in order to record by this method an accurate velocity curve of the blood flow within a blood vessel, it is necessary 1, to place the vessel in a constant and uniform magnetic field with its axis at right angles to the lines of magnetic force; 2, to attach electrodes to the vessel wall at diametrically opposite points in a line perpendicular to both the axis of the vessel and the lines of magnetic force; and 3, to keep the cross sectional area of the vessel constant in the plane of the electrodes. It was found that the blood vessel wall was a sufficiently good electrical conductor, so that the electrodes could be placed on its surface without piercing the wall. The electrodes can now be connected to a suitable recording system and the resulting variations in potential recorded will give a true picture without lag of the velocity variations of the blood flow within the vessel.

*Apparatus.* In the experiments herein reported the constant magnetic field was obtained with a large electromagnet reconstructed from a magnet kindly given to us by the Cambridge Instrument Company of New York. When energized with 12 volts D.C., the field strength between the pole pieces N, S was approximately 20,000 oersted. The magnet was mounted on a rigid support in such a manner that it could be raised or lowered, and rotated about a horizontal and a vertical axis. The distance between the pole pieces could be adjusted by means of a screw; the pole tips were insulated with thin rubber finger cots to avoid artefacts caused by the metallic pole pieces touching the artery, the electrodes, or any surrounding tissue. A much smaller magnet was used successfully in some experiments. Its field strength, however, was only one-seventh that of the larger magnet, so that the flow potentials set up were much smaller. With a more powerful amplifier than we used, however, even smaller and weaker magnets would be usable.

The connections of the complete flowmeter are shown diagrammatically in figure 11. The artery to be used was held in place between the poles of the magnet by means of a small transparent bakelite sleeve ($S$, fig. 11). The artery was first exposed and stripped of superficial fascia, and then slipped into the sleeve through the horizontal slot in its side. The sleeve containing the artery was held in position by placing it between the magnet poles and then tightly approximating them. The two non-polarizable




Figs. 11–14

Fig. 11. Diagram of connections of complete flowmeter used in these experiments. *Art.*, artery in sleeve; *S*, bakelite sleeve; *W*, agar-saline soaked wick; *B*, porous boot; *Z*, amalgamated Zn strip; *Comp.*, compensating circuit consisting of battery, reversing switch, and resistances; *F*, electrically driven tuning fork; $C_1$, contact in fork driving circuit; *R*, resistance across $C_1$ for eliminating spark and resulting interference; $C_2$, adjustable contact, insulated from fork, for interrupting input circuit; *Amp.*, amplifier; *G*, oscillograph; *L*, light source; *C*, camera. The two poles of the magnet are omitted. They are placed in front and behind the bakelite sleeve.

Fig. 12. Calibration curve of a pulsating flow through an excised carotid artery.

Fig. 13. Calibration curve of a pulsating flow through the carotid artery of an anesthetized dog.

Fig. 14. Diagram of circuit of the A.C. method. *P*, *P*, poles of electromagnet energized with alternating current; *E*, *E*, electrodes; *C*, compensating coil, phase control; *V*, potentiometer, voltage control. Discussed in text.

electrodes consisted of short woolen wicks, *W*, soaked in an agar-saline solution and attached to porous clay boots, *B*, which were filled with saturated zinc sulfate solution into which dipped amalgamated zinc strips, *Z*. The free ends of the wicks were inserted in two vertical holes in the top and bottom of the bakelite sleeve flush with the internal cylindric wall so that they made contact with the wall of the artery. The size of the hole in the sleeve is such that the artery is slightly constricted; this insures good contact between the electrodes and the vessel wall at all times, and likewise maintains the diameter of the artery constant.

The flow potentials are recorded by means of an amplifier and oscillograph (kindly given to us by the General Electric X-Ray Corporation of Chicago). The amplifier, *Amp.*, is composed of three stages resistance-capacity coupled, and has a voltage gain of 500. It responds to frequencies as low as 1 or 2 per sec. with negligible distortion. The oscillograph, *G*, is a moving iron vane type with a natural frequency of about 100 vibrations per second. Due to the type of interstage coupling, the amplifier will amplify only alternating or pulsating currents; therefore, in order to record the absolute flow in the vessel, whether constant or pulsating, the input circuit is interrupted 50 times per second by an electrically driven tuning fork (*F*, fig. 2). The oscillograph then records a pulsating current, oscillating between zero and a peak value which is a function of the potential difference at the artery. If the blood flow is constant, this peak value is constant, while if the flow is pulsating, the pulsating current is modulated accordingly. A reversal of flow will produce a reversal of deflections with respect to the zero-line. The contact, $C_2$, on the interrupter must be adjusted so that the duration of the make is equal to the duration of the break; at this point the maximum amplitude of the pulsating current is obtained, and on the photographic record the light intensity of the zero line is equal to the light intensity of the flow line, i.e., the peaks of the pulsations

In practice, there are always spurious constant electromotive forces set up at the points of contact of the electrodes with the artery, so that even when there is no blood flow, the galvanometer will record a deflection above or below zero. For this reason a compensating battery circuit (*comp.*, fig. 11) is introduced into the input. With the blood flow stopped by clamping the artery peripherally to the sleeve (or with the electromagnet turned off), the compensating current is varied until the galvanometer shows no deflection from zero. Thus the spurious galvanic potential is exactly neutralized.

*Calibration.* The response of the amplifier is linear over a wide range, i.e., the deflection of the galvanometer is directly proportional to the input voltage. As shown above by equation (2), the input voltage is a linear function of the velocity of blood flow, hence the calibration curve of the

instrument is a straight line passing through zero. This was proven experimentally on an excised artery with an artificial circulation system by comparing the mean deflection of the flowmeter with the actual mean flow as measured with a graduate and stopwatch. The resulting curve is shown in figure 12, and this was also verified in the living animal (cf. fig. 13). To calibrate then, it is necessary to determine only one other point on the curve. This is easily and accurately done in vivo as follows: The artery is clamped off peripherally to the sleeve, and the compensating current is adjusted for zero deflection of the galvanometer. An 18 or 20 gauge needle is then introduced in the vessel between the sleeve and the clamp, and a measured quantity of blood (viz., 10 cc.) is slowly drawn into the syringe and then rapidly reinjected. The mean deflection corresponding to the injection of 10 cc. is determined by measuring with a planimeter the area enclosed between the zero line and the flow curve from the beginning to the end of the deflection and dividing this area by its length along the horizontal or time axis (cf. fig. 2 E). This mean deflection in millimeters then represents a velocity, namely, $\frac{10 \text{ cc.}}{t}$ per second (which when divided by the mean deflection gives the rate of flow represented by a deflection of 1 scale division). The error of this determination can be reduced to about 1 per cent by repeating this procedure four times. The calibration curve plotted as millimeters deflection against velocity in cubic centimeters per second can be quickly constructed by drawing a straight line from zero through the point just determined. Since the external diameter of the artery is fixed by the sleeve, the internal diameter may be found by subtracting from this the double thickness of the vessel wall, found by measuring the flattened artery with a micrometer. Knowing the internal vessel diameter, one may calculate the internal cross-sectional area and thus convert velocity in cubic centimeters per second to linear velocity in centimeters per second.

*The alternating field method.* The foregoing method includes two inconvenient features: first a D.C. amplifier has to be used or else the input of an A.C. amplifier has to be interrupted, and secondly, nonpolarizable electrodes have to be employed. Both these requirements are avoided by the alternating current method described below and twice the sensitivity is obtained with the same peak value of the magnetic field. Equation (1) will assume a more general meaning if we imagine that $H$ is variable in time, $H\ (t)$, and if we add $A$ to the right side of the equation:

$$E = H\ (t) \cdot l \cdot v \cdot 10^{-8} + A \text{ volts.} \qquad (3)$$

This last equation represents the alternating voltage which will be induced in the streaming liquid when the magnet is energized with alternating current. By putting $v = 0$ we see that there is an induced voltage $A$ even

when the fluid is at rest. This voltage is induced in the loop formed by the blood vessel and the lead wires by the periodically growing and collapsing magnetic field. The member $H\ (t) \cdot l \cdot v \cdot 10^{-8}$ is entirely due to motion of the liquid. Since in practice we are observing not the instantaneous value of $E$ but rather the peak of the oscillograph deflection, it will be more useful to consider:

$$E\ max. = H\ max.\ l \cdot v \cdot 10^{-8} + A. \tag{4}$$

Then with a certain velocity $v$ we shall obtain a certain maximum deflection of the recording beam determined by equation (4), the two borderlines of the deflection being symmetrical with respect to the base-line. The reason for this is the periodic change in direction of the magnetic field so that $E\ max.$ changes its sign with $H\ max.$ The total deflection thus corresponds to $2\ E\ max.$ The preceding description was given on the assumption of $A = 0$. In reality, however, $A$ in some cases will be even greater than the deflection due to flow, and there is no simple addition possible of the alternating voltage due to flow and the one represented by $A$ because of a phase difference. But fortunately, it is easily possible to eliminate $A$, the voltage induced when the liquid is at rest. Figure 14 illustrates the method by which this can be accomplished. $A$ of the equation above can be reduced to a minimum by the use of non-inductive leads from the blood vessel. For complete elimination a "compensating coil," $C$, with a potentiometer, $V$, is introduced in one of the leads, its orientation and position in the stray magnetic field being fixed so that the A.C. voltage induced in it by the stray field is opposite to the voltage expressed by $A$. This opposed voltage can be made equal in magnitude to the voltage $A$ by adjustment of potentiometer $V$ (voltage control).[4] In this way it is possible to exactly neutralize $A$ and to obtain zero deflection for zero flow, and the flowmeter equation for alternating field assumes the simple form:[5]

$$E\ max. = H\ max.\ l \cdot v \cdot 10^{-8} \text{ volt}. \tag{5}$$

As the current generated by flow is alternating, the electrodes do not have to be nonpolarizable, polarization being prevented by the rapid change of polarity at the electrodes. In practice we used the bakelite sleeve described above. In its electrode channels thick copper wires were inserted halfway, the remaining space being filled with a saline clay paste

[4] The position of coil $C$ with respect to the magnet is of importance to ensure the proper phase relationship between the voltage to be compensated and the compensating voltage.

[5] The linear relationship of calibration with the A.C. method using tap water in an artificial circulation model using an excised artery was demonstrated with an average error of 0.3 per cent.

since bare electrodes in contact with the vessel give rise to spurious deflections of the oscillograph. It is desirable to make the magnet core out of laminated transformer iron as this greatly reduces heating by eddy currents, thereby permitting the use of greater currents to energize the magnet. A resistance capacitance coupled amplifier of the simplest design can be used with this arrangement, and the interruption of input becomes superfluous. It is obvious that direction of flow cannot be indicated by the A.C. method.

*Precautions to avoid artefacts in the D.C. method.* Contact of metals with the blood vessel in the neighborhood of the electrodes or with the leads should be avoided to prevent the creation of spurious potential differences. It is, therefore, advisable to insulate the poles of the magnet by rubber. This we found to be more durable than lacquer. Careful shielding is desirable to reduce amplifier disturbances. Fluctuations of the line voltage may be a source of inaccuracy if the magnet voltage is supplied from a power line. We avoided this source of error by using a 12 volt storage battery.

The blood vessel in the sleeve should be somewhat constricted, otherwise a sharp drop of blood pressure might cause a passive narrowing of the vessel sufficient to break the contact with the electrodes. This is the most likely cause of disturbance if the sleeve does not constrict the vessel sufficiently. With a tightly fitted blood vessel the layer of moisture on the surface of the blood vessel is reduced to a negligible thickness so that no appreciable variations of sensitivity due to variations in surface moisture could occur. An excessive layer of surface moisture would decrease the sensitivity of the flowmeter since it would provide a low resistance shunt across the electrodes in addition to the shunt path already offered by the artery wall. The resistance of this latter path, however, is constant in any one case, and sufficiently high to be relatively unimportant.

In any flow of liquid through a tube, the velocity is greatest in the center and slower near the walls of the tube, due to viscosity. Thus the flow electromotive forces set up along the diameter of the artery will be greater in the central portion than at the periphery, but the sum of infinitesimal potential differences induced in various portions of the diameter, which is recorded by the flowmeter, represents, as can be shown mathematically, the average velocity of the fluid in question. Thus the flowmeter records the mean instantaneous velocity at the cross-section where it is applied.

If the magnetic field penetrating the blood vessel is strictly uniform, eddies should not create erroneous results because only the velocity component at right angles to the lines of force and the electrode axis will contribute to the recorded voltage. To secure a homogeneous field the height of the pole pieces should exceed the diameter of the vessel sufficiently.

SUMMARY

A method is described for measuring cyclic variations in the velocity of blood flow in unopened blood vessels based on the induction of an e.m.f. in the blood when moving through a magnetic field. Cyclic variations in the velocity of blood flow through the carotid artery of anesthetized dogs were recorded with this D.C. method under various conditions, viz: *1*, with the anesthetized animal intact; *2*, with the chest opened; *3*, during rapid saline infusion; *4*, during partial occlusion and after release of the thoracic aorta; *5*, during aortic valve insufficiency in which an early diastolic back flow was demonstrated; *6*, during marked cardiac arrhythmia; *7*, during ectopic beats, and *8*, during gasping respiration. The characteristics of the velocity curves in each case are described and their significance discussed.

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# THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY


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VOL. 122—NO. 3

*Issued June 1, 1938*

BALTIMORE, U. S. A.

1938

Entered as second-class matter, August 18, 1914, at the Post Office in Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918

*Made in United States of America*

# THE AMERICAN JOURNAL OF PHYSIOLOGY

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